APPENDIX E

U.S. Patent No. 4,795,756;

U.S. Patent No. 6,100,271;

U.S. Patent No. 6,103,730;

U.S. Patent No. 6,316,444 (front page and columns 101-102) ;

U.S. Patent No. 6335,324 (front page and columns 27-30, 39-293; and

U.S. Patent No. 6,376,472

United States Patent [19]

Oxford et al.

[11] **Patent Number: 4,795,756**

[45] **Date of Patent: Jan. 3, 1989**

[54] 3-(2-AMINOETHYL)INDOLE DERIVATIVES

[75] Inventors: **Alexander W. Oxford**, Royston; **Michael D. Dowle**, Ware, both of England

[73] Assignee: **Glaxo Group Limited**, London, England

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[52] **U.S. Cl.** **514/415**; 548/504; 564/157; 514/930

[58] **Field of Search** 514/415; 548/504

[56] **References Cited**

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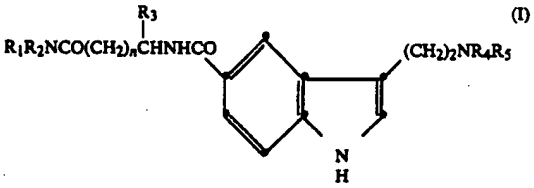
Primary Examiner—Mark L. Berch
Attorney, Agent, or Firm—Bacon & Thomas

[57] **ABSTRACT**

Compounds are disclosed of formula (I)



wherein

R_1 is H, C_{1-6} alkyl, C_{3-7} cycloalkyl, phenyl optionally substituted by C_{1-3} alkoxy or phen(C_{1-4}) alkyl in which the phenyl ring is optionally substituted by C_{1-3} alkoxy;

R_2 is H or C_{1-6} alkyl;

R_3 is H or C_{1-3} alkyl;

R_4 and R_5 independently represents H, C_{1-3} alkyl or 2-propenyl; and

n represents zero or 1;

and physiologically acceptable salts and solvates (e.g. hydrates) thereof.

The compounds have potent selective vasoconstrictor activity and are indicated as useful for the treatment of migraine. The compounds may be formulated as pharmaceutical compositions with physiologically acceptable carrier or excipients for administration by any convenient route. Various methods for the preparation of the compounds (I) are disclosed.

11 Claims, No Drawings

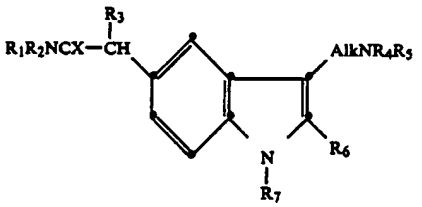
3-(2-AMINOETHYL)INDOLE DERIVATIVES

This invention relates to indole derivatives, to processes for their preparati n, to pharmaceutical compositions containing them and to their medical use, in particular to compounds and compositions of use in the treatment of migraine.

The pain of migraine is associated with excessive dilatation of the cranial vasculature, and known treatments for migraine include the administration of compounds having vasoconstrictor properties, such as ergotamine. However, ergotamine is a non-selective vasoconstrictor which constricts blood vessels throughout the body and has undesirable and dangerous side effects. Migraine may also be treated by administering an analgesic, usually in combination with an antiemetic, but such treatments are of limited value.

There is thus a need for a safe and effective drug for the treatment of migraine, which can be used either prophylactically or to alleviate an established headache, and a compound having a selective vasoconstrictor activity would fulfil such a role.

A number of classes of compounds have been described having selective vasoconstrictor activity including, for example, the indole derivatives described in our UK Patent Specification No. 2082175 of formula:



wherein R_1, R_3, R_4, and R_6 and R_7, which may be the same or different, each represents a hydrogen atom or an alkyl group;

R_2 represents a hydrogen atom or an alkyl, aryl, aralkyl, cycloalkyl or alkenyl group, or R_1 and R_2 together with the nitrogen atom to which they are attached form a saturated monocyclic 5 to 7-membered ring which may optionally contain a further heterofunction;

R_5 represents a hydrogen atom or an alkyl or alkenyl group, or R_4 and R_5 together form a aralkylidene group;

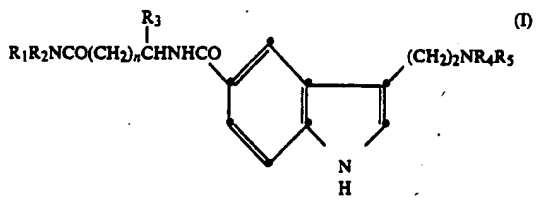
Alk represents an alkylene chain containing two or three carbon atoms which may be unsubstituted or substituted by not more than two C_{1-3} alkyl groups;

X represents an oxygen or sulphur atom; and physiologically acceptable salts and solvates thereof.

As indicated in UK Patent Specification No. 2082175 compounds of the above formula selectively constrict the carotid arterial bed of the anaesthetised dog and are thus potentially useful for the treatment of migraine.

We have now found a n vel group of indole derivatives having potent and selective vasoconstrictor activity.

Thus, the present invention provides an indole of the general formula (I):



(I)

wherein

R_1 represents a hydrogen atom, a C_{1-16} alkyl group, a C_{3-7} cycloalkyl group, a phenyl group which may be unsubstituted or substituted by a C_{1-3} alkoxy group or a phen(C_{1-4}) alkyl group in which the phenyl ring may be unsubstituted or substituted by a C_{1-3} alkoxy group;

R_2 represents a hydrogen atom or a C_{1-6} alkyl group;

R_3 represents a hydrogen atom or a C_{1-3} alkyl group;

R_4 and R_5 which may be the same or different each represents a hydrogen atom, a C_{1-3} alkyl group or 2-propenyl; and

n represents zero or 1; and physiologically acceptable salts and solvates (e.g. hydrates) thereof.

The invention includes within its scope all optical isomers of compounds of formula (I) and their mixtures, including the racemic mixtures thereof.

Referring to the general formula (I), the alkyl group may be straight chain or branched chain alkyl groups, such as methyl, ethyl or prop-2-yl groups. A cycloalkyl group represented by R_1 may be for example a cyclopentyl or cyclohexyl group.

When R_1 represents a substituted phenyl or substituted phen(C_{1-4})alkyl group the C_{1-3} alkoxy substituent may be for example methoxy.

The alkyl moiety of the phen(C_{1-4})alkyl group may be for example a methyl or ethyl moiety.

A preferred class of compounds represented by the general formula (I) is that in which R_1 represents a hydrogen atom, a C_{1-6} alkyl group, for example a methyl group, a phenyl group or a phen(C_{1-4})alkyl group, for example a phenylmethyl group.

In the compounds of formula (I), n is preferably zero.

Another preferred class of compounds of formula (I) is that wherein R_2 represents a hydrogen atom. A further preferred class of compounds is that in which R_3 represents a hydrogen atom.

Another preferred class of compounds of formula (I) is that in which R_4 and R_5, which may be the same or different each represents a hydrogen atom or a methyl group.

A still further preferred class of compounds falling within the scope of formula (I) is that wherein R_1 represents a hydrogen atom or a phenylmethyl group, R_2 and R_3 both represent a hydrogen atom, R_4 represents a hydrogen atom or a methyl group, R_5 represents a methyl group and n is zero.

Preferred compounds according to the invention include:

N-(2-Amino-2-oxoethyl)-3-[2-(methylamino)ethyl]-1H-indole-5-carboxamide;

N-(2-Amino-2-oxoethyl)-3-[2-(dimethylamino)ethyl]-1H-indole-5-carboxamide;

and physiologically acceptable salts and solvates thereof.

Suitable physiologically acceptable salts of the indoles of general formula (I) include acid addition salts formed with inorganic or organic acids, for example hydrochlorides, hydrobromides, sulphates, nitrates,

phosphates, tartrates, citrates, fumarates, maleates, succinates, and sulph nates e.g. mesylates. Other salts may be useful in the preparation of compounds of formula (I) e.g. creatinine sulphate adducts.

It will be appreciated that the inventi n extends to other physiologically acceptable equivalents of the compounds according to the invention, i.e. physiologically acceptable compounds which are converted in vivo into the parent compound. Examples of such equivalents include physiologically acceptable, metabolically labile N-acyl derivatives.

Compounds of the invention selectively constrict the carotid arterial bed of the anaesthetised dog, whilst having a negligible effect on blood pressure. Their selective vasoconstrictor action has been demonstrated in vitro.

Compounds of the invention are useful in treating pain resulting from dilatation of the carotid vascular bed, in particular migraine and cluster headache.

Accordingly, the invention also provides a pharmaceutical composition adapted for use in human medicine which comprises at least one compound of formula (I) or a physiologically acceptable salt or solvate (e.g. hydrate) thereof and formulated for administration by any convenient route. Such compositions may be formulated in conventional manner using one or more pharmaceutically acceptable carriers or excipients.

Thus the compounds according to the invention may be formulated for oral, buccal, parenteral or rectal administration or in a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium starch glycollate); r wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may taken the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g. sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agents (e.g. lecithin or acacia); non-aqueous vehicles (e.g. almond oil, oily esters or ethyl alcohol); and preservatives (e.g. methyl or propyl-p-hydroxybenzoates or sorbic acid). The liquid preparations may also contain conventional buffers, flavouring, colouring and sweetening agents as appropriate.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds of the invention may be formulated for parenteral administration by injection. Formulations for injection may be presented in unit dosage form e.g. in ampoules or in multi-dose containers, with an added preservative.

The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

The compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

For administration by inhalation the compounds according to the invention are conveniently delivered in the form of an aerosol spray presentation from pressurised packs, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas, or from a nebuliser. In the case of a pressurised aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

A proposed dose of the compounds of the invention for oral, parenteral, buccal or rectal administration to man (of average bodyweight e.g. about 70 kg) for the treatment of migraine is 0.1 to 100 mg of the active ingredient per unit dose which could be administered, for example, 1 to 4 times per day. It will be appreciated that it may be necessary to make routine variations to the dosage depending on the age and weight of the patient as well as the severity of the condition to be treated. The dosage will also depend on the route of administration.

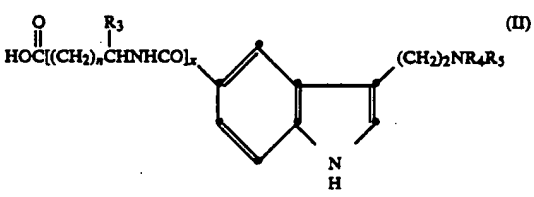
For oral administration a unit dose will preferably contain from 2 to 50 mg of the active ingredient. A unit dose for parenteral administration will preferably contain 0.2 to 5 mg of the active ingredient.

Aerosol formulations are preferably arranged so that each metered does or 'puff' delivered from a pressurised aerosol contains 0.2 to 2 mg of a compound of the invention and, each dose administered via capsules or cartridges in an inhaler or insufflator contains 0.2 to 20 mg. The overall daily dose by inhalation will be within the range 1 mg to 100 mg. Administration may be several times daily, for example from 2 to 8 times, giving for example 1, 2 or 3 doses each time.

The compounds of the invention may, if desired, be administered in combination with one or more other therapeutic agents, such as analgesics, anti-inflammatory agents and anti-nauseants.

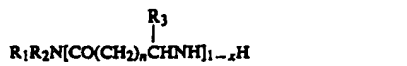
According to another aspect of the invention, compounds of formula (I), and physiologically acceptable salts or solvates (e.g. hydrates) thereof, may be prepared by the general methods outlined below. In the following processes, R_1, R_2, R_3, R_4, R_5 and n are as defined for the general formula (I) unless otherwise specified.

According to one general process (A), a compound of general formula (I) may be prepared by reacting an acid of general formula (II):



(II)

(wherein) x is zero or 1) or an acylating derivative thereof (e.g. an acid halide, anhydride or an ester), or a salt (for example an organic or inorganic acid addition salt such as the hydrochloride, hydrobromide, sulphate or maleate salt, or creatinine sulphate adduct) or a protected derivative thereof, with an appropriate amine of general formula (III):



(III)

or a salt (e.g. hydrochloride) thereof.

The above reaction is preferably effected using an activated derivative of formula (II).

Activated derivatives of general formula (II) which may be employed in the preparation of compounds of formula (I) include acid anhydrides (e.g. mixed anhydrides such as pivalic anhydride or diphenyl carbamic anhydride or formed with a sulphonyl halide such as methanesulphonyl chloride, or a haloformate such as a lower alkylhaloformate); esters (e.g. methyl, ethyl,p-nitrophenyl or 1-methlpyridinium ester); and acid halides (e.g. acid chlorides).

When using an activated derivative of general formula (II) the condensation process may be effected in aqueous or non-aqueous reaction media and conveniently at a temperature of from −70° to +150° C. Thus the condensation reaction using an alkyl ester may be effected in a suitable reaction medium such as an alcohol e.g. methanol; an amide e.g. dimethylformamide; an ether e.g. tetrahydrofuran or diethylether; or mixtures thereof and conveniently at a temperature of from 0° to 100° C. The condensation reaction using an acid halide, anhydride or activated ester may be effected in a suitable reaction medium such as an amide e.g. N,N-dimethylformamide; an ether e.g. tetrahydrofuran or diethylether; a nitrile e.g. acetonitrile; a halogenated hydrocarbon e.g. dichloromethane; or mixtures thereof preferably at a temperature of from −5° to +30° C. The condensation reaction may if desired be carried out in the presence of a base, such as a tertiary amine (e.g. triethylamine or pyridine); or an inorganic base such as an alkali metal carbonate (e.g. potassium carbonate) or bicarbonate (e.g. sodium bicarbonate). A tertiary amine base such as pyridine may also act as the reaction solvent. In some instances, for example when x is 1, the amine of general formula (III) may itself act as the reaction solvent.

If desired, the above condensation reactions may be carried out in the presence of a catalyst such as 4-dimethylaminopyridine.

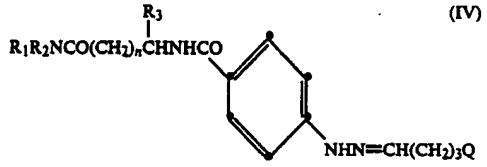
When an acid of general formula (II) is employed, the reaction is desirable conducted in the presence of a coupling agent, for example N,N'- carbonyldiimidazole or a carbodiimide such as N,N'-dicyclohexyl-carbodiimide. The condensation reaction may be carried out in a suitable reaction medium preferably an anhydrous medium, conveniently at a temperature of from −50° to +50° C., preferably −5° to +30° C. Suitable solvents include halogenated hydrocarbons e.g. dichloromethane; nitriles e.g. acetonitrile; amides e.g. N,N-dimethylformamide; and ethers e.g. tetrahydrofuran; as well as mixtures of two or more such solvents. The reactiion may also be carried out in the absence of a coupling agent in a suitable reaction medium such as a hydrocarbon (e.g. t luene or xylene) conveniently at a temperature of from 50° to 120° C.

Where it is desired to prepare a compound of formula (I) in which R_1 and R_2 are both hydrogen atoms the condensation may be effected using ammonia, which may be for example be employed in the form of aqueous ammonia or in a solvent such as methanol.

Acids and activated derivatives of formula (II) wherein x is zero may be prepared as described for example in UK Published Patent Application No. 2035310. Compounds of formula (II) wherein x is 1 may be prepared by analogous methods. Activated derivatives of general formula (II) wherein x is 1 may also be prepared by reacting an activated derivative of formula (II) wherein x is zero with an amino acid ester of formula $R'CO(CH_2)_nCH(R_3)NH_2$, (wherein R' represents an alkoxy group, preferably a C_{1-6} alkoxy group) or a salt (e.g. a hydrochloride) thereof, as described for general process (A) itself.

The intermediate compounds of general formula (II) wherein x is 1 and the acylating derivatives thereof, are novel compounds and constitute a further feature of this invention.

According to another general process (B), compounds of formula (I) may be prepared by the cyclisation of a compound of general formula (IV):



(IV)

wherein Q is the group NR_4R_5 (or a protected derivative thereof) or a leaving atom or group such as a halogen atom (e.g. chlorine or bromine) or an acyloxy group (e.g. a carboxylic or sulphonic acyloxy group such as an acetoxy, chloroacetoxy, dichloroacetoxy, trifluoroacetoxy, p-nitrobenzoyloxy, p-toluenesulphonyloxy or methanesulphonyloxy group).

The reaction may conveniently be effected in aqueous or non-aqueous reaction media, and at temperature of from 20° to 200° C., preferably 50° to 125° C.

Particularly convenient embodiments of the process are described below.

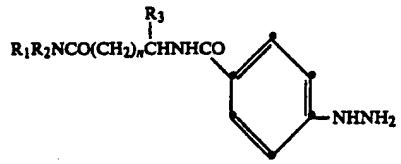
When Q is the group NR_4R_5 (or a protected derivative thereof) the process is desirably carried out in the presence of polyphosphate ester in a reaction medium which may comprise one or more organic solvents, preferably halogenated hydrocarbons such as chloroform, dichloromethane, dichloroethane, dichlorodifluoromethane, or mixtures thereof. Polyphosphate ester is a mixture of esters which may be prepared from phosphorus pentoxide, diethylether and chloroform according to the method described in 'Reagents for Organic Synthesis', (Fieser and Fieser, John Wiley and Sons 1967).

Alternatively the cyclisation may be carried out in an aqueous or non-aqueous reaction medium, in the presence of an acid catalyst. When an aqueous medium is employed this may be an aqueous organic solvent such as an aqueous alcohol (e.g. methanol, ethanol or isopropanol) or as aqueous ether (e.g. dioxan or tetrahydrofuran) as well as mixtures of such solvents and the acid catalyst may be, for example, an inorganic acid such as concentrated hydrochloric or sulphuric acid. (In some cases the acid catalyst may also act as the reaction sol-

vent). In an anhydrous reaction medium, which may comprise one or more alcohols or ethers (e.g. as previously described) r esters (e.g. ethyl acetate), the acid catalyst will generally be a Lewis acid such as boron trifluoride, zinc chloride or magnesium chloride.

When Q is a leaving atom or group such as a chlorine or bromine atom the reaction may be effected in an aqueous organic solvent, such as an aqueous alcohol (e.g. methanol, ethanol or isopropanol) or an aqueous ether (e.g. dioxan or tetrahydrofuran) in the absence of an acid catalyst, conveniently at a temperature of from 20° to 200° C., preferably 50° to 125° C. This process results in the formation of a compound of formula (I) wherein R_4 and R_5 are both hydrogen atoms.

According to a particular embodiment of general process (B) compounds of formula (I) may be prepared directly by the reaction of a compound of general formula (V):



(V)

or a salt thereof,
with a compund of formula (VI):

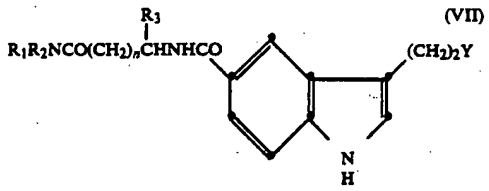
$$OHC(CH_2)_3Q \quad (VI)$$

(wherein Q is as defined above) or a salt or protected derivative thereof (such as an acetal or ketal e.g. formed with an appropriate alkyl orthoformate or diol, or protected as a bisulphite addition complex) using the appropriate conditions as described above for the cyclisation of compounds of general formula (IV). It will be appreciated that in this embodiment of the cyclisation process (B) a compound of general formula (IV) is formed as an intermediate, and may either be isolated prior to cyclisation or reacted in situ to form the desired compound of general formula (I).

Compounds of general formula (IV) may, if desired, be isolated as intermediates during the process for the preparation of compounds of formula (I) wherein a compound of formula (V), or a salt or protected derivative thereof, is reacted with a compound of formula (VI), or a salt or protected derivative thereof, in a suitable solvent, such as an aqueous alcohol (e.g. methanol) at a temperature of, for example, 20° to 30° C. If an acetal or ketal of a compound of formula (VI) is used, it may be necessary to carry out the reaction in the presence of an acid (for example, acetic or hydrochloric acid).

Compounds of general formula (V) may be prepared for example from the corresponding nitro compounds, using conventional procedures.

A further general process (C) f r preparing compounds of general formula (I) inv lves reacting a compound of general formula (VII):



(VII)

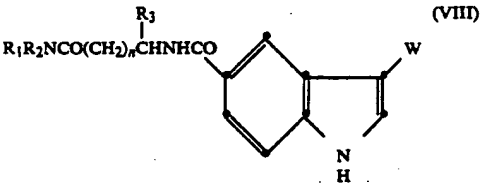
(wherein Y is a readily displaceable atom or group) or a protected derivative thereof, with an amine of formula R_4R_5NH.

The displacement reaction may conveniently be carried out on those compounds of formula (VII) wherein Y is a halogen atom (e.g. chlorine, bromine or iodine) or a group OR_6 where OR_6 is, for example, an acyloxy group which may be derived from a carboxylic or sulphonic acid, such as an acetoxy, chloroacetoxy, dichloroacetoxy, trifluoroacetoxy, p-nitrobenzoyloxy, p-toluenesulphonyloxy or methanesulphonyloxy group.

The displacement reaction may be conveniently effected in an inert organic solvent (optionally in the presence of water), examples of which include alcohols, e.g. ethanol; cyclic ethers, e.g. dioxan or tetrahydrofuran; acylic ethers e.g. diethylether; esters, e.g. ethyl acetate; amides, e.g. N,N-dimethylformamide; and ketones e.g. acetone or methylethyl ketone, at a temperature of from −10° to +150° C., preferably 20° to 50° C.

The compounds of general formula (VII) wherein Y is a halogen atom may be prepared by reacting a hydrazine of general formula (V) with an aldehyde or ketone (or a protected derivative thereof) of formula (VI) in which Q is a halogen atom, in an aqueous alkanol (e.g. methanol) containing an acid (e.g. acetic or hydrochloric acid). Compounds of formula (VII) wherein Y is the group OR_6 may be prepared from the corresponding compound wherein Y is a hydroxyl group by acylation with the appropriate activated species (e.g. anhydride or sulphonyl chloride) using conventional techniques. The intermediate alcohol may be prepared by cyclisation of a compound of formula (IV) wherein Q is a hydroxyl group (or a protected derivative thereof) under standard conditions.

Compounds of formula (I) may also be prepared by another general process (D) involving reduction of a compound of general formula (VIII):



(VIII)

(wherein W is a group capable of being reduced to give the required $-(CH_2)_2NR_4R_5$ group or to give a protected derivative of the $-(CH_2)_2NR_4R_5$ group) or a salt or protected derivative thereof.

The required $-(CH_2)_2-$ and $-NR_4R_5$ groups may be formed by reduction steps which take place separately or together in any appropriate manner.

Groups which may be reduced to the $-(CH_2)_2-$ moiety include the the corresponding unsaturated group and corresponding groups containing one or more carbonyl functions and/or a hydroxyl group.

Groups which may be reduced to the group $-NR_4R_5$ where R_4 and R_5 are both hydrogen include nitr , azido, hydroxyimino and nitrile groups. In the latter case, reduction yields the group $-CH_2NH_2$ and thus provides a methylene group of the $-(CH_2)_2-$ moiety.

The required $-NR_4R_5$ group wherein R_4 and/or R_5 other than hydrogen may be prepared by reduction of a nitrile $-CH_2CN$ or an aldehyde $-CH_2CHO$ in the presence of an amine, R_4R_5NH.

A particularly suitable method for preparing a compound of formula (I) wherein R_4 and/or R_5 is other than hydrogen is reductive alkylation of the corresponding compound wherein R_4 and/or R_5 represent hydrogen with an appropriate aldehyde or ketone (e.g. formaldehyde or acetone) in the presence of a suitable reducing agent. In some instances (e.g. for the introduction of the group(s) R_4 and/or R_5, where these represent methyl) the aldehyde (e.g. formaldehyde) may be condensed with the amine and the intermediate thus formed may subsequently be reduced using a suitable reducing agent.

Examples of groups represented by the substituent W thus include $-(CH_2)_2NO_2$; $-CH=CHNO_2$; $-(CH_2)_2N_3$; $-CH_2CN$; $-CH_2CHO$; $-COCH_2Z$; $-CH_2CH=NOH$; and $-CH(OH)CH_2NR_4R_5$ (wherein Z is an azido group or the group $-NR_4R_5$ or a protected derivative thereof).

The reduction according to general process (D) may be effected by conventional methods, for example, by catalytic hydrogenation or using a reducing agent such as an alkali metal or alkaline earth metal borohydride or cyanoborohydride, or a metal hydride. The reduction may conveniently be effected in an organic reaction medium, which may comprise one or more solvents and at temperatures between $-20°$ and $+150°$ C. Suitable solvents include alcohols e.g. ethanol or propanol; cyclic ethers e.g. dioxan or tetrahydrofuran; acyclic ethers e.g. diethylether; amides e.g. dimethylformamide; esters e.g. ethyl acetate; and nitriles e.g. acetonitrile.

It will be appreciated that the choice of reducing agent and reaction conditions will be dependent on the nature of the group W, and other groups already present on the molecule.

Suitable reducing agents which may be used in the above process for the reduction of compounds of formula (VIII) wherein W represents, for example, the groups $-(CH_2)_2NO_2$, $-CH=CHNO_2$, $-(CH_2)_2N_3$, $-CH_2CN$, $-CH_2CH=NOH$ and $-CH(OH)CH_2NR_4R_5$ include hydrogen in the presence of a metal catalyst, for example Raney Nickel or a noble metal catalyst such as platinum, platinum oxide, palladium, palladium oxide or rhodium, which may be supported, for example, on charcoal, kieselguhr or alumina. In the case of Raney Nickel, hydrazine may also be used as the source of hydrogen. This process may conveniently be carried out in a solvent such as an alcohol e.g. ethanol; an ether, e.g. dioxan or tetrahydrofuran, an amide, e.g. dimethylformamide; or an ester e.g. ethyl acetate, and at a temperature of from $-10°$ to $+50°$ C., preferably $-5°$ to $+30°$ C.

The reduction process may also be effected on compounds of formula (VIII) wherein W represents, for example, the gr ups $-(CH_2)_2NO_2$, $-CH=CHNO_2$, $-(CH_2)_2N_3$, $-CH(OH)CH_2NR_4R_5$ or $-COCH_2Z$ (where Z is as previously defined), using an alkali metal or alkaline earth metal borohydride or cyanoborohydride e.g. sodium or calcium borohydride or cyanoborohydride which process may conveniently be carried out in an alcohol such as propanol or ethanol, or a nitrile such as acetonitrile, and at a temperature of from 10° to 100° C., preferably 50° to 100° C. In some instances the reduction using a borohydride may be carried out in the presence of cobaltous chloride.

Reductive alkylation of a compound of formula (VIII) may be effected using an alkali earth metal borohydride or cyanoborohydride. The reaction may be effected in an aqueous or non-aqueous reaction medium, conveniently in an alcohol (e.g. methanol or ethanol) or an ether (e.g. dioxan or tetrahydrofuran) optionally in the presence of water. The reaction may conveniently be carried out at a temperature in the range 0° to 100° C., preferably 5° to 50° C.

A particular embodiment of general process (D) is the reduction of a compound of formula (VIII) wherein W is the group $-CH_2CN$, for example by catalytic reduction with hydrogen in the presence of a catalyst such as palladium on charcoal or rhodium on alumina, optionally in the presence of an amine HNR_4R_5. The reduction may be effected in a suitable solvent such as an alcohol, e.g. methanol or ethanol.

A compound of general formula (I) where R_5 is a hydrogen atom may also be prepared by reduction of a corresponding compound wherein R_5 is a benzyl group, e.g. with hydrogen in the presence of a catalyst, e.g. 10% palladium on carbon.

The starting materials or intermediate compounds of formula (VIII) wherein W represents $-(CH_2)_2NO_2$, $-CH=CHNO_2$, $-CH_2CN$ or $-COCH_2Z$ may be prepared by analogous methods to those described in UK Published Patent Application No. 2035310, and 'A Chemistry of Heterocyclic Compounds—Indoles Part II', Chapter VI, edited by W J Houlihan (1972) Wiley Interscience, New York.

Compounds of formula (VIII), wherein W is the group $-CH_2CHO$ may be prepared by oxidation (e.g. with Jones' reagent) of a compound of formula (VII) wherein Y is a hydroxyl group. A compound of formula (VIII) wherein W is the group $-CH_2CH=NOH$ may be prepared by treatment of the corresponding aldehyde with hydroxylamine hydrochloride using standard conditions.

The intermediate compound of formula (VIII) wherein W is the group $-(CH_2)_2N_3$ may be prepared from a compound of formula (VIII) wherein Y is a halogen atom using standard procedures.

Standard reducing agents such as sodium borohydride may be used to prepare a compound of formula (VII) wherein W is the group $-CH(OH)CH_2NR_4R_5$ from the corresponding compound of formula (VIII) wherein W is the group $-COCH_2NR_4R_5$.

According to a further general process (E) a compound of formula (I) according to the invention, or a salt or protected derivative thereof, may be converted into another compound of formula (I) using conventional procedures.

For example, a compound of general formula (I) wherein one or more of R_1, R_2, R_4 and R_5 are alkyl groups may be prepared from the corresponding compounds of formula (I) wherein one or more of R_1, R_2, R_4 and R_5 represent hydrogen atoms, by reduction with a suitable alkylating agent such as a compound of formula R_xL, (where R_x represents the desired R_1, R_2, R_4 or R_5 group and L represents a leaving atom or group such as a halogen atom or a tosylate group) or a sulphate $(R_x)_2SO_4$. Thus, the alkylating agent may be for

example an alkyl halide (e.g. methyl or ethyl iodide), alkyl tosylate (e.g. methyl tosylate) r dialkylsulphate (e.g. dimethylsulphate).

The alkylation reaction may conveniently be carried ut in an inert organic solvent such as an amide (e.g. dimethylf rmamide), an ether (e.g. tetrahydrofuran) r an aromatic hydrocarbon (e.g. toluene) preferably in the presence of a base. Suitable bases include, for example, alkali metal hydrides such as sodium or potassium hydride; alkali metal amides such as sodium amide; alkali metal carbonates such as sodium carbonate; alkali metal alkoxides such as sodium or potassium methoxide, ethoxide or t-butoxide; and tetrabutylammonium fluoride. When an alkyl halide is employed as the alkylating agent the reaction may also be carried out in the presence of an acid scavenging agent such as propylene or ethylene oxide. The reaction may be conveniently effected at a temperature of from −20° to +100° C.

Compounds of formula (I) wherein R_1 representss a cycloalkyl or phenylalkyl group and/or one or both of R_4 and R_5 represents propenyl may be prepared similarly, using an appropriate compound of formula R_xL or $(R_x)_2SO_4$.

According to another general process (F), a compound of general formula (I) according to the invention, r a salt thereof may be prepared by subjecting a protected derivative of general formula (I) or a salt thereof to reaction to remove the protecting group or groups.

Thus, at an earlier stage in the reaction sequence for the preparation of a compound of general formula (I) or a salt thereof it may have been necessary or desirable to protect one or more sensitive groups in the molecule to av id undesirable side reactions. For example it may be necessary to protect the group NR_4R_5, wherein R_4 and/or R_5 represents hydrogen, by protonation or with a group easily removable at the end of the reaction sequence. Such groups may include, for example, aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl; or acyl groups such as N-benzyloxycarbonyl, t-butoxycarbonyl or phthaloyl.

In some cases, it may also be desirable to protect the indole nitrogen with, for example, an aralkyl group such as benzyl.

Subsequent cleavage of the protecting group or groups may be achieved by conventional procedures. Thus an aralkyl group such as benzyl, may be cleaved by hydrogenolysis in the presence of a catalyst (e.g. palladium on charcoal) or sodium and liquid ammonia; an acyl group such as N-benzyloxycarbonyl may be removed by hydrolysis with, for example, hydrogen bromide in acetic acid or by reduction, for example by catalytic hydrogenation. The phthaloyl group may be removed by hydrazinolysis (e.g. by treatment with hydrazine hydrate) or by treatment with a primary amine (e.g. methylamine).

As will be appreciated, in some of the general processes (A) to (E) described previously it may be necessary or desirable to protect any sensitive groups in the molecule as just described. Thus, a reaction step involving deprotection of a protected derivative of general formula (I) or a salt thereof may be carried out subsequent to any of the previously described processes (A) to (E).

Thus, according to a further aspect of the invention, the following reactions in any appropriate sequence may if necessary and/or desired by carried out subsequent to any of the processes (A) to (E):

(i) removal of any protecting groups; and

(ii) conversion of a compound of general formula (I) or a salt thereof into a physiologically acceptable salt or solvate (e.g. hydrate) thereof.

Where it is desired to isolate a c mpound of the invention as a salt, f r example as an acid additi n salt, this may be achieved by treating the free base of general formula (I), with an appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

The starting materials or intermediate compounds for the preparation of the compounds according to this invention may be prepared by analogous methods to those described in UK Published Patent Application No. 2035310.

As well as being employed as the last main step in the preparative sequence, the general methods indicated above for the preparation of the compounds of the invention may also be used for the introduction of the desired groups at an intermediate stage in the preparation of the required compound. Thus, for example, the required group at the 5-position may be introduced before or after cyclisation to form the indole nucleus. It should therefore be appreciated that in such multi-stage processes, the sequences of reactions should be chosen in order that the reaction conditions do not affect groups present in the molecule which are desired in the final product.

The invention is further illustrated by the following Examples. All temperatures are in ° C.

Chromatography was carried out either in the conventional manner using silica gel (Merck, Kieselgel 60, Art. 7734) or by flash chromatography on silica (Merck 9385) and thin layer chromatography (t.l.c.) on silica (Macherly-Nagel, Polygram) except where otherwise stated.

Intermediates were routinely checked for purity by t.l.c. employing u.v. light for detection and spray reagents such as potassium permanganate ($KMnO_4$). In addition indolic intermediates were detected by spraying with aqueous ceric sulphate (CeIV) and tryptamines by spraying with a solution of iodoplatinic acid (IPA) or ceric sulphate.

The following abbreviations define the eluents used for column chromatography and t.l.c.:

(A) Ethyl acetate-isopropanol-water-0.88 ammonia	25:15:8:2
(B) Chloroform-methanol	19:1
(C) Methylene chloride-ethanol-0.88 ammonia	50:8:1
(D) Methylene chloride-ethanol-0.88 ammonia	25:8:1

Proton (^1H) nuclear magnetic resonance (n.m.r.) spectra were obtained either at 90 MHz using a Varian EM 390 instrument or at 250 MHz using a Bruker AM or WM 250 instrument. s=singlet, d=doublet, t=triplet, m=multiplet and q=quartet.

INTERMEDIATE 1

Methyl 3-[(dimethylamino)methyl]-1H-indole-5-carboxylate hydrochloride

A solution of methyl 1H-indole-5-carboxylate (0.24 g) in dry acetonitrile (15 ml) containing N,N-dimethyl)methylene)ammonium chl ride (0.13 g) was stirred at room temperature, under nitrogen, for 5 h. The resulting precipitate was filtered ff, and dried in vacuo at room temperature overnight to give the *title compound* as a solid (0.3 g) m.p. 197°–199°.

INTERMEDIATE 2

Methyl 3-(cyanomethyl)-1H-indole-5-carboxylate

Methyl iodide (2.3 ml) was added portionwise to a stirred soluti n of Intermediate 1, as the free base (7.8 g) in dry dimethylsulphoxide (50 ml). The resulting suspension was stirred at room temperature for 30 min, potassium cyanide (11.0 g) added, and the suspension stirred at room temperature for 18 h. The suspension was then partitioned between water (500 ml) and ethyl acetate (2×200 ml). The combined extracts were washed with water (200 ml) dired ($MgSO_4$) and evaporated in vacuo. The residue was purified by 'flash' chromatography (B) to give a solid (2.4 g). A sample (0.5 g) was crystallised from a mixture of ethyl acetate and hexane to give the *title compound* as a solid (0.35 l g) m.p. 127°–129°.

INTERMEDIATE 3

3-(Cyanomethyl)-1H-indole-5-carboxylic acid

A suspension of Intermediate 2 (1.8 g) in a mixture of methanol (30 ml) and sodium hydroxide (2N, 15 ml) was stirred at room temperature for 3 days. The methanol was evaporated in vacuo, and the residue partitioned between hydrochloric acid (2N, 50 ml) and ethyl acetate (2×50 ml). The combined extracts were dried ($MgSO_4$) and evaporated in vacuo to give a solid which was crystallised from ethyl acetate to give the *title compound* as a solid. (1.0 g) m.p. 248°–250° (decomp.)

INTERMEDIATE 4

3-[2-(Dimethylamino)ethyl]-1H-indole-5-carboxylic acid hydrochloride

A suspension of 10% palladised charcoal (0.4 g) in ethanol (10 ml) was stirred under an atmosphere of hydrogen until uptake ceased. To the catalyst was added a solution of Intermediate 3 (0.45 g) in 33% ethanolic dimethylamine (20 ml) and the mixture was again stirred under an atmosphere of hydrogen until all the starting material had been consumed. The suspension was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in ethanol (10 ml) and the solution was treated with ethanolic hydrogen chloride until it was just acidic. Evaporation of the solvent gave a gum which crystallised from propan-2-ol (15 ml) as a powder (0.35 g), m.p. 200–1°. An analytical sample crystallised from ethanol had m.p. 205–6°.

EXAMPLE 1

N-(2-Amino-2-oxoethyl)-3-(2-aminoethyl)-1H-indole-5-carboxamide hydrochloride compound with ethanol and water, 5:5:1:1

(i) Phenylmethyl 2-[5-[[(2-amino-2-oxoethyl)amino]carbonyl]-1H-indol-3-yl]ethyl carbamate

A mixture of phenylmethyl 2-[5-[[[(diphenylamino)-carbonyl]oxo]-carbonyl]-1H-indol-3-yl]ethyl carbamate (Intermediate 5, prepared as in British Specification No. 2035310) (4.0 g), glycinamide hydrochloride (1.1 g) and sodium acetate (1.4 g) in dimethylformamide (50 ml) was stirred f r 3 h at room temperature. The soluti n was then partitioned between saturated sodium chloride (200 ml) and ethyl acetate (200 ml), and the aqueous phase further extracted with ethyl acetate (100 ml). The combined organic extracts were washed with water (3×100 ml), and dried ($MgSO_4$). Removal of the solvent gave a solid (3.2 g). Chromatography on silica gel, eluting with 0–5% methanol in chloroform, afforded a solid (1.5 g) which was recrystallised from aqueous ethanol to give the *title compound* as a crytalline solid, m.p. 185°–186° (0.75 g).

(ii) N-(2-Amino-2-oxoethyl)-3-(2-aminoethyl)-1H-indole-5-carboxamide hydrochloride compound with ethanol and water (5:5:1:1)

The product of Stage (i) (0.55 g) in ethanol (90 ml) was hydrogenated over palladium oxide on charcoal (5%, 0.24 g) for 3 h at room temperature and atmospheric pressure. The catalyst was removed by filtration and washed with ethanol (2×30 ml). The filtrates were combined and concentrated to a clear glass, which was taken up in ethanol (2 ml), treated with ethereal hydrogen chloride (6 ml) and diluted with ether (30 ml). The solid that formed was washed with ether (2×20 ml) and dried at 60°/0.4 torr. for 18 h to give the *title compound* as an amorphous solid m.p. 170°–185° (0.34 g).

Analysis found : C, 51.9; H, 6.0; N, 18.0. $C_{13}H_{16}N_4O_2.HCl\ 0.2C_2H_5OH\ 0.2H_2O$ requires : C, 52.0; H, 6.0; N, 18.1%.

N.m.r. δ(DMSO-d6) includes 3.00 (4H, br s, CH_2CH_2N), 3.90 (2H, d, $N—CH_2—C=O$), 7.07 (1H, s, $CONH_2$ (one)), 8.75 (1H, br t, $CONH$-CH_2) and 11.3 (1H, br s, indole —NH).

EXAMPLE 2

N-[2-(Methylamino)-2-oxoethyl]-3-(2-aminoethyl)-1Hindole-5-carboxamide hydrochloride compound with ethanol and water 10:10:3:3

(i) Methyl [[[3-[2-[[(phenylmethoxy)carbonyl]amino]ethyl]-1H-indol-5-yl]carbonyl]amino]acetate

Intermediate 5 (3.3 g), methyl glycinate hydrochloride (1.55 g) and sodium acetate (1.23 g) were stirred together at room temperature in dimethylformamide for 1 h. The mixture was partitioned in dimethylformamide for 1 h. The mixture was partitioned between ethyl acetate (150 ml) and water (150 ml), and the aqueous layer further extracted with ethyl acetate (150 ml). The combined organic extracts were washed with water (2×100 ml), and dried (Na_2SO_4). The solvent was evaporated in vacuo to give an oil (4.1 g) which was chromatographed on silica using a nmixture of 0–2% methanol in chloroform as eluent. The appropriate fractions were calculated, and the solvent was evaporated in vacuo to give a solid (2.0 g). Recrystallisation from isopropyl acetate gave the *title compound* (1.5 g) as microcrystals m.p. 118°–119°.

(ii) Phenylmethyl 2-[5-[[[2-(methylamino)-2-oxoethyl]amino]carbonyl]-1H-indol-3-yl]ethyl carbamate

The product of Stage (i) (1.0 g) was stirred in 33% solution of methylamine in ethanol (5 ml) at room temperature for 5 min. The solvent was evaporated in vacu and the solid was triturated with hot ethyl acetate. The product was filtered off and dried in vacuo to give the *title compound* (0.96 g), m.p. 178°–180°.

(iii)
N-[2-(methylamino)-2-oxoethyl]-3-(2-aminoethyl)-1H-indole-5-carboxamide hydrochloride compound with ethan 1 and water 10:10:3:3

A solution of the product of Stage (i) (0.95 g) in ethanol (120 ml) was hydrogenated over 10% palladium on charcoal (0.4 g) for 1 h. The catalyst was filtered off, and washed with ethanol (50 ml). The filtrate was evaporated in vacuo to give a clear glass, which was dissolved in ethanol (5 ml), and ethereal hydrogen chloride (2 ml) was added. The mixture was diluted with dry ether (80 ml), and the resultant solid was filtered off. The solid was washed with ether (2 ×15 ml) and dried at 60° C./0.4 torr for 16 h to give the title compound (0.66 g), m.p. 110°–120°.

Analysis found : C,53.5; H,6.2; N,16.8; $C_{14}H_{18}N_4O_2.HCl.0.3C_2H_6O.0.3H_2O$ requires : C,53.1; H,6.5; N,17.0%

N.m.r. δ(DMSO-d6) includes 2.65 (3H, d, $\underline{CH_3}$—NH), 3.0 (4H, br s, $\underline{CH_2CH_2N}$) 3.90 (2H, d, $\underline{CH_2}$—NH—C=O) 8.70 (1H, br t, CH_2—$\underline{NH}$—C=O) and 11.3 (1H, br s, indole —NH).

EXAMPLE 3

N-(2-Amino-2-oxo-1-methylethyl)-3-(2-aminoethyl)-1H-indole 5-carboxamide hydrochloride compound with ethanol and water (10:10:4:1)

(i) Ethyl 2-[[[3-[2-[[(phenylmethoxy) carbonyl]amino]ethyl]-1H-indol-5-yl]carbonyl]amino]-propanoate

Intermediate 5 (6.0 g), D,L-alanine ethyl ester (3.8 g) and potassium carbonate (3.5 g) were stirred together in dimethylformamide (30 ml) at room temperature for 4 h. The mixture was partitioned between ethyl acetate (150 ml) and water (150 ml), and the aqueous phase further extracted with ethyl acetate (150 ml). The organic extracts were combined and washed with water (2×100 ml). The organic phase was dried (Na_2SO_4) and the solvent evaporated in vacuo to give a solid which was chromatographed on silica using ethyl acetate as eluent. The appropriate fractions were combined, and the solvent evaporated in vacuo to give a solid. Recrystallisation from isopropyl acetate gave the *title compound* as a microcrystalline solid (2.5 g), m.p. 143°–144°.

(ii) Phenylmethyl 2-[5-[[[2-(1-amino-1-oxo)propyl]amino]carbonyl]-1H-indol-3-yl]ethyl carbamate

The product of Stage (i) (1.0 g) was stirred in a mixture of aqueous ammonia (0.88, 60 ml) and methanol (80 ml) for 16 h. The mixture was partitioned between ethyl acetate (100 ml) and hydrochloric acid (150 ml). The organic layer was separated, dried (Na_2SO_4) and the solvent was evaporated in vacuo to give a semi-solid (1.1 g), which was chromatographed on silica (50 g) using ethyl acetate as eluent. The appropriate fractions were combined, and the solvent was evaporated in vacuo to give a colourless glass (0.7 g). The *title compound* crystallised from ethyl acetate as a solid (0.5 g), m.p. 163°–165°.

(iii)
N-(2-Amino-2-oxo-1-methylethyl)-3-(2-aminoethyl)-1H-indole-5-carboxamide hydrochloride compound with ethanol and water (10:10:4:1)

A solution of the product of Stage (ii) (0.35 g) in ethanol (50 ml) was hydrogenated over 10% palladium on carbon (0.2 g) at room temperature and atmospheric pressure for 2 h. The catalyst was filtered off, and washed with ethanol (2×20 ml). The combined filtrates were evaporated in vacuo to give a clear glass (0.21 g) which was dissolved in ethanol (4 ml) and treated with ethereal hydrogen chloride (1 ml). The mixture was diluted with dry ether (100 ml) and stirred for 10 min. The resulting solid was filtered off, washed with ether (2×20 ml) and dried at 60° C./0.4 torr for 4 h to give the *title compound* as a solid (0.18 g), m.p. 135°–145°.

Analysis found : C,53.6; H,6.4; N,16.7; $C_{14}H_{18}N_4O_2.HCl.0.4C_2H_6O.0.1H_2O$ requires : C,53.7; H,6.6; N,16.9%.

N.m.r δ(DMSO-d6) includes 1.40 (3H, d, $\underline{Ch_3}$-CH), 3.05 (4H, br s, $\underline{CH_2CH_2N}$) 4.55 (1H, qui, CH_3-$\underline{CH}$) and 11.4 (1H, br s, indole —NH).

EXAMPLE 4

N-(2-Amino-2-oxoethyl)-3-[2-(methylamino)ethyl]-1H-indole-5-carboxamide hydrochloride compound with ethanol and water (10:10:1:3)

(i)
N-(2-Amino-2-oxoethyl)-3-[2-(phenylmethylamino)ethyl]-1H-indole-5-carboxamide

hydrochloride compound with ethanol (5:5:1)

The product of Example 1 (as the free base) (1.3 g) and benzaldehyde (0.53 g) were dissolved in absolute ethanol (20 ml) and stirred at room temperature for 20 h. Sodium borohydride (0.19 g) was then added in portions over 10 min. The solution was stirred for an additional 15 min, and the solvent was evaporated in vacuo. The residue was dissolved in dilute hydrochloric acid, and the solution basified with sodium hydrogen carbonate (2N, 40 ml). The solution was saturated with potassium carbonate, and extracted with ethyl acetate (7 ×50 ml). The organic extracts were combined, dried (Na_2SO_4) and the solvent evaporated in vacuo to give a glass (1.67 g). A small portion of this product (0.25 g) was dissolved in ethanol (3 ml), and ethereal hydrogen chloride (1 ml) was added. The mixture was diluted with ether (40 ml), and the resulting solid was filtered, washed with ether (2×30 ml), and dried at 60° C./0.5 torr for 16 h to give the title compound (0.14 g) m.p. 105°–120°.

Analysis found : C,61.9; H,6.2; N,14.2; $C_{20}H_{22}N_4O_2.HCl.0.2C_2H_6O$ requires : C,61.9; H,6.2; N,14.2%.

(ii)
N-(2-Amino-2-oxoethyl)-3-[2-(methylamino)ethyl]-1H-indole-5-carboxamide hydrochloride compound with ethanol and water (10:10:1:3)

Dimethyl sulphate (0.26 g) was added to the product of Stage (i) (0.68 g) and potassium carbonate (0.5 g) in dimethylformamide with stirring. After 4 h the mixture was partitioned between ethyl acetate (150 ml) and water (150 ml), and the aqueous phase further extracted with ethyl acetate (50 ml). The combined organic extracts were washed with water (2×100 ml), dried (Na_2SO_4), and evaporated in vacuo to give the N-methyl derivative as an oil (0.38 g). [T.l.c. (A) Rf. 0.59].

A solution of the above product (0.32 g) in ethanol (30 ml) was hydrogenated over 10% palladium on charcoal (0.2 g) at room temperature and one atmosphere pressure for 6 h. (Hydrogen uptake 25 ml). The catalyst was filtered off, and washed with ethanol (3 ml), and

ethereal hydrogen chloride (1 ml) was added. The mixture was diluted with ether (30 ml), and the resulting solid filtered off, washed with ether (2×20 ml), and dried in vacuo to give the *title compound* as a solid (0.13 g), m.p. 100°–110°.

Analysis found : C,53.1; H,6.2; N,17.1; $C_{14}H_{18}N_4O_2.HCl.0.1C_2H_6O.0.3H_2O$ requires : C,53.2; H,6.4; N,17.5%.

N.m.r. δ(DMSO-d6) includes 2.50 (3H, t, NH—$\underline{CH_3}$), 3.10 (4H, br s, $\underline{CH_2CH_2N}$), 3.80 (2H, d, $\underline{CH_2}$—NH—C=O), 8.70 (1H, br t, CO$\underline{NH}$-CH_2) and 11.2 (1H, br s, indole —NH).

EXAMPLE 5

N-(3-Amino-3-oxopropyl)-3-(2-aminoethyl)-1H-indole-5-carboxamide, hydrochloride compound with ethanol and water (4:4:1:5).

(i) Phenylmethyl 2-[5-[[(3-amino-3-oxopropyl)amino]carbonyl]-1H-indol-3-yl]ethyl carbamate

Intermediate 5 (5.0 g) and beta-alanine ethyl ester hydrochloride (3.0 g) were stirred together in dimethylformamide (25 ml) for 1.5 h. The mixture was partitioned between ethyl acetate (150 ml) and water (150 ml) and the aqueous phase was further extracted with ethyl acetate (100 ml). The combined organic extracts were washed with water (2×150 ml), dried ($MgSO_4$) and evaporated in vacuo to give an oil (5.0 g). This was chromatographed twice on silica eluting with 0.1% methanol in chloroform, and then ethyl acetate. The appropriate fractions were combined, and the solvent was removed in vacuo to give the ester as an oil (2.6 g) which was dissolved in methanolic ammonia (240 ml), and stirred at room temperature for 158 h. Solvent was removed in vacuo to give a solid (2.4 g) which was crystallised from aqueous ethanol to give the *title compound* as a microcrystalline solid (1.6 g), m.p. 169°–170°.

(ii) N-(3-Amino-3-oxopropyl)-3-(2-aminoethyl)-1H-indole-5-carboxamide hydrochloride compound with ethanol and water (4:4:1:5)

A solution of the product of Stage (i) (0.5 g) in ethanol (100 ml) was hydrogenated over 10% palladium on carbon (0.3 g) at room temperature and on atmosphere pressure for 18 h. The catalyst was filtered off, and washed with ethanol (2×15 ml). The combined filtrates were evaporated in vacuo to give a clear oil (0.4 g) which was dissolved in absolute ethanol (4 ml), and treated with ethereal hydrogen chloride (1 ml). The mixture was diluted with dry ether (50 ml) and stirred for 10 min. The resulting solid was filtered off, washed with ether, and dried in vacuo at 60° for 6 h to give the *title compound* as a hygroscopic solid (0.32 g).

Analysis found : C,50.1; H,6.5; N,15.8; $C_{14}H_{18}N_4O_2.HCl,0.25C_2H_6O.1.25H_2O$ requires : C,50.5; H,6.7; N,16.3%.

N.m.r. δ(DMSO-d6) includs 2.50 (2H, t, $\underline{COCH_2}$—CH_2), 3.15 (4H, br s, $\underline{CH_2CH_2}$—NH_2) 3.50 (2H, q, $COCH_2$-$\underline{CH_2}$) and 11.4 (1H, br s, indole —NH).

EXAMPLE 6

3-(2-Aminoethyl)-N-[2-oxo-2-(phenylamino)ethyl]-1H-indole-5-carboxamide hydrochloride hydrate

(i) Phenylmethyl 2-[5-[[[2-oxo-2-(phenylamino)ethyl]amino]carbonyl]-1H-indol-3-yl]ethyl carbamate

A solution of 3-[2-[[(Phenylmethoxy)carbonyl]amino]ethyl]-1H-indole-5-carboxylic acid (1.35 g) in dry tetrahydrofuran (27 ml), was stirred, under nitrogen and cooled to between −10° C and −5° C. Triethylamine (0.809 g) and methanesulphonyl chloride (0.5 g) were added nand stirring continued for 1 hr. 2-Amino-N-phenylacetamide (0.60 g) and 4-dimethylaminopyridine (0.097 g) in dry tetrahydrofuran (10 ml) were added and the resulting mixture was allowed to warm to room temperature. The reaction mixture was partitioned between hydrochloric acid (2N; 100 ml) and ethyl acetate (2×100 ml). The combined organic extracts were washed with sodium carbonate (2N; 100 ml), dried ($MgSO_4$) and evaporated in vacuo to give a foam (1.24 g). Flash chromatography (B) gave the product as a solid (0.69 g), which was crystallised from ethanol to give the *title compound* as a solid (0.35 g) m.p. 202.5°–203.5°.

(ii) 3-(2-Aminoethyl)-N-[2-oxo-2-(phenylamino)ethyl]-1H-indole-5-carboxamide hydrochloride hydrate

A solution of the product of Stage (i), (0.30 g) in ethanol (50 ml) was added to pre-reduced 10% palladium oxide on charcoal (0.1 g; 50% aqueous paste) and ethanol (10 ml) and the resulting mixture hydrogenated at room temperature and pressure for 4 h. The catalyst was filtered off through 'hyflo' and the filtrate evaporated in vacuo to give an oil, which was dissolved in absolute alcohol (10 ml), and acidified with ethanolic hydrogen chloride. This solution was diluted with dry ether until the hydrochloride salt precipitated out. This was filtered off and dried in vacuo to give the *title compound* as a solid. (82.5 mg) m.p. 188°–191° C.

Analysis Found : C,58.3; H,5.7; N,13.7; $C_{19}H_{20}N_4O_2.HCl.H_2O$ requires : C,58.3; H,5.9; N,14.3%.

N.m.r. δ(DMSO-d6) includes 3.15 (4H, m. $\underline{CH_2CH_2}$—N),4.15 (2H, d, CO$\underline{CH_2}$-N), 10.2 (1H, s, CO$\underline{NH}$-Ph) and 11.3 (1H, br s, indole —NH).

EXAMPLE 7

N-(2-Amino-2-oxoethyl)-3-[2-(dimethylamino)ethyl]-1H-indole-5-carboxamide compound with ethanol and water

(i) Methyl [[[3-(cyanomethyl)-1H-indol-5-yl]carbonyl]amino]acetate

A stirred suspension of Intermediate 3 (1.973 g) in anhydrous tetrahydrofuran (100 ml) was treated with N,N-carbonyldiimidazole (1.74 g) and stirred at room temperature for 0.5 h. The suspension was heated under reflux for 0.75 h and then stirred at room temperature for 2 h. Triethylamine (1.36 ml), and glycine methyl ester hydrochloride (1.24 g) were added and the suspension stirred for 20 h at room temperature. A further portion of triethylamine (0.68 ml) and glycine methyl ester hydrochloride (0.62 g) was added and stirring continued at room temperature for another 5 h. The

suspension was evaporated to dryness and the residue mixed thoroughly with 1N hydrochloric acid (100 ml) and extracted with ethyl acetate (8×200 ml). The combined organic extracts were washed with 8% sodium hydrogen carbonate (100 ml), dried ($MgSO_4$) and evaporated to dryness to afford a gum (1.05 g). This material was chromatographed on silica eluting with cyclohexane, cyclohexane/isopropyl acetate mixtures and isopropyl acetate.

Appropriate fractions were evaporated and triturated with dry ether to present the *title compound* as a powder (0.448 g) m.p. 138°–140°.

(ii)

N-(2-Amino-2-oxoethyl)-3-(cyanomethyl)-1H-indole-5-carboxamide

The product of Stage (i) (0.05 g) dissolved in methanolic ammonia (2 ml) was stirred overnight, further 2 ml portions of methanolic ammonia being added after 3.5 h and 5.5 h. The solution was evaporated to dryness and the residual solid triturated with anhydrous ether to present the product as a powder (0.036 g) m.p. 206°–209°.

Assay Found: C,60.4; H,4.8; N,21.4; $C_{13}H_{12}N_4O_2.0.1CH_3OH$ requires C,60.6; H,4.8; N,21.6%.

(iii)

N-(2-Amino-2-oxoethyl)-3-[2-(dimethylamino)ethyl]-1H-indole-5-carboxamide

A suspension of the product of Stage (ii) (0.192 g) in 33% ethanolic dimethlamine (20 ml) was added to a slurry of pre-reduced 10% palladium oxide on carbon (0.3 g of a 50% paste with water) in ethanol (10 ml). The resultant mixture was hydrogenated at room temperature and atmospheric pressure for 24 h. Subsequent filtration (to remove the catalyst) and evaporation of the solvent left a gum (0.216 g), which was chromatographed on silica (C and D). Evaporation of the appropriate fractions afforded a colourless glass (0.197 g) which was triturated with anhydrous ether to present the *title compound* free base as a powder (0.144 g).

Water Assay Found: 0.95%. Theory 1.35%.

Analysis Found: C,60.7;H,7.7;N,18.1. $C_{15}H_{20}N_4O_2.0.33EtOH.0.23H_2O$ requires C,61.1;H,7.35;N,18.2%.

N.m.r. δ(DMSO-d6) includes (2.20 (6H, s, N—Me_2), 2.58 (2H, t, CH_2—CH_2—NMe_2), 3.85 (2H, d, NH—CH_2-C=O), 8.55 (1H, t, CONH-CH_2) and 11.10 (1H, br s, indole —NH).

EXAMPLE 8

3-[2-(Dimethylamino)ethyl]-N-[2-oxo-2-[(phenylmethyl)amino]ethyl]-H-indole-5-carboxamide oxalate hydrate

A suspension of Intermediate 4 (0.2 g) in dry pyridine (4 ml) at −5° was treated with thionyl chloride (0.064 ml) and stirred for 40 min. 2-Amino- N-benzylacetamide (0.1222 g) dissolved in dry pyridine (4 ml) was added (at −5°) and the solution allowed to stand at room temperature for 60 h. Evaporation of the pyridine afforded a gum (0.35 g) which was chromatographed on silica (C and D). Evaporation of the appropriate fractions gave the free base as a partially crystalline gum (0.084 g). A soluti n of the free base (0.063 g) in absolute alcohol (1 ml) was treated with a solution of xalic acid (15 mg) in absolute alcoh l (0.5 ml). The resultant suspension was diluted with absolute alcohol (1 ml) and the solid was filtered off and washed with absolute alcohol (2 ml) to give a powder (54 mg) m.p. 165°–170°.

Water Assay Found 1.31% H_2O. Theory (for $\frac{1}{3}H_2O$) = 1.26% Assay Found : C,60.5;H,5.7;N,11.8.

$C_2H_{26}N_4O_2.C_2H_2O_4.\frac{1}{3}H_2O$ requires C,60.75;H,6.1;N,11.8%.

N.m.r δ(DMSO-d6) includes 2.80 (6H, s, N—Me_2), 3.17 (4H, AA'BB', CH_2CH_2-N), 3.97 (2H, d, NH—CH_2-C=O), 4.35 (2H, d, NH-CH_2-Ph), 8.70 (1H, t, CONH—CH_2—C=O) and 11.3 (1H, s, indole —NH).

The following examples illustrates a pharamaceutical formulation according to the invention containing N-(2-amino-2-oxoethyl)-3-[2-(methylamino) ethyl]-1H-indole-5-carboxamide hydrochloride as the active ingredient. Other compounds of the invention may be formulated in a similar manner.

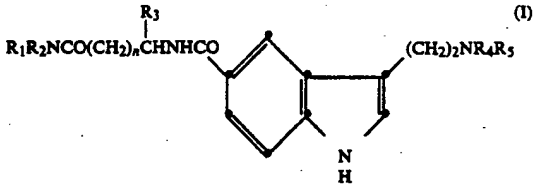
Tablets for Oral Administration

	mg/tablet
Active Ingredient	100
Magnesium stearate BP	1.0
Anhydrous lactose	99

The active ingredient is sieved and blended with the anhydrous lactose and magnesium stearate. The mix is then compressed into tablets using a Manesty F3 tablet machine fitted with 8.0 mm concave punches.

We claim:

1. A compound selected from compouns of the formula (I):



(I)

wherein

R_1 represents a hydrogen atom, a C_{1-6} alkyl group, a C_{3-7} cycloalkyl group, a phenyl group which may be unsubstituted or substituted by a C_{1-3} alkoxy group or a phen(C_{1-4}) alkyl group in which the phenyl ring may be unsubstituted or substituted by a C_{1-3} alkoxy group;

R_2 represents a hydrogen atom or a C_{1-6} alkyl group;

R_3 represents a hydrogen atom or a C_{1-3} alkyl group;

R_4 and R_5 which may be the same or different each represents a hydrogen atom, a C_{1-3} alkyl group or 2-propenyl; and

n represents zero or 1;

and physiologically acceptable salts and solvates thereof.

2. A compound according to claim 1, wherein, in the formula (I), R_1 represents a hydrogen atom, a C_{1-6} alkyl group, a phenyl group or a phen(C_{1-4})alkyl group.

3. A compound according to claim 1, wherein, in the formula (I), n represents zero.

4. A compound according to claim 1, wherein, in the formula (I), R_2 represents a hydrogen atom.

5. A compound according to claim 1, wherein, in the formula (I), R_3 represents a hydr gen atom.

6. A compound according to claim 1, wherein, in the formula (I), R_4 and R_5 which may be the same or different, each represents a hydrogen atom or a methyl group.

7. A compound according to claim 1, wherein, in the formula (I), R_1 represents a hydrogen atom or a phenylmethyl group, R_2 and R_3 each represents a hydrogen atom, R_4 represents a hydrogen atom or a methyl group, R_5 represents a methyl group and n is zero.

8. A compound according to claim 1, selected from N-(2-amino-2-oxoethyl)-3-[2-(methylamino) ethyl-1H-indole-5-carboxamide;

N-(2-amino-2-oxoethyl)-3-[2-(dimethylamino) ethyl]-1H-indole-5-carboxamide;

and the physiologically acceptable salts and solvates thereof.

9. A pharmaceutical composition which comprises at least one compound selected from compounds of formula (I) as defined in claim 1 and physiologically acceptable salts and solvates thereof together with a pharmaceutically acceptable carrier or excipient therefor.

10. A method of treating a patient susceptible to or suffering from migraine which comprises administering to the patient a pharmaceutical composition as claimed in claim 9.

11. A method of treating a patient susceptible to or suffering from migraine which comprises administering to the patient an effective amount of a compound of formula (I) as defined in claim 1 or a physiologically acceptable salt or solvate thereof.

* * * * *

United States Patent **6,100,271**
Klein , et al. **August 8, 2000**

Therapeutic compounds containing xanthinyl

Abstract

Therapeutic compounds with at least one carboxylic acid, ester or amide-substituted side chain have the formula: CORE MOIETY --(R).sub.j wherein j is an integer from one to three. The core moiety is non-cyclic or cyclic (carbocyclic or heterocyclic). R may be selected from among hydrogen, halogen, hydroxyl, amino, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, carbocyclic or heterocyclic groups and at least one R has the formula I: ##STR1## wherein: one or two p are the integer one, otherwise p is two; and n is an integer from three to twenty; R.sub.1 is selected from the group consisting of substituted and unsubstituted CH.sub.2 ; NR.sub.3, R.sub.3 being hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or C.sub.(1-20) hydroxyalkyl, or carbocyclic or heterocyclic group; O; --CHR.sub.4 O--, R.sub.4 being substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl, or R.sub.2 and R.sub.4 join to form a substituted or unsubstituted heterocycle having four to seven ring atoms, the ether group --O-- of --CHR.sub.4 O-- being a member of the heterocycle. R.sub.2 is selected from the group consisting of hydrogen; halogen; substituted or unsubstituted C.sub.(1-10) alkyl; C.sub.(1-10) alkoxyl; C.sub.(2-10) alkenyl; C.sub.(1-10) hydroxyallyl; --A(R.sub.5).sub.m, A being N or O, m being one or two and R.sub.5 being hydrogen, a substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(1-10) alkoxyl, C.sub.(2-10) alkenyl or C.sub.(1-10) hydroxyalkyl), or carbocyclic or heterocyclic group. At least one of R.sub.1 is NR.sub.3, O or --CHR.sub.4 O--, or R.sub.2 is --A(R.sub.5).sub.m. The compounds and pharmaceutical compositions thereof are useful as therapies for diseases advanced via intracellular signaling through specific intracellular signaling pathways by mediating a signaling response to an external stimuli.

Inventors: **Klein; J. Peter** (Vashon, WA); **Leigh; Alistair J.** (Brier, WA); **Underiner; Gail E.** (Brier, WA); **Kumar; Anil M.** (Seattle, WA)

Assignee: [redacted]

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Intern'l Class: A61K 031/522; C07D 473/10

Field f Search: 544/271,268,269 514/263,265

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Primary Examiner: [redacted]
Attorney, Agent or Firm: [redacted]

Parent Case Text

CROSS REFERENCE TO RELATED APPLICATION

This is a Continuation-in-Part patent application of U.S. patent application Ser. No. 08/199,368, which was filed on Feb. 18, 1994 and which is now abandoned.

Claims

What is claimed is:

1. A therapeutic compound, including resolved enantiomers, diastereomers, salts, o[redacted] having the formula:

CORE MOIETY --(R).sub.j

wherein:

j is an integer from one to three;

the core moiety is xanthinyl; and

R is a member selected from the group consisting of hydrogen, hydroxyl, amino, C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, carbocyclic group and heterocyclic group and at least one R having formula I: ##STR8## with the proviso that the R having formula I is not attached at the N.sub.9 nitrogen atom of the xanthinyl;

wherein:

p is two;

n is an integer from seven to twenty;

(CH.sub.p).sub.n is unsubstituted or substituted by a member selected from the group consisting of halogen, hydroxyl, C.sub.(1-10) allyl, C.sub.(2-10) alkenyl, C.sub.(1-10) alkoxyl, C.sub.(1-10) alkanoyloxyl, C.sub.(1-10) oxoalkyl, carbocyclic group and heterocyclic group;

R.sub.1 is a member selected from the group consisting of CH.sub.2 ; NR.sub.3, R.sub.3 being hydrogen, C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl, or carbocyclic or heterocyclic group; O; --CHR.sub.4 O-- or --C(R.sub.4).sub.r O--, r being one or two, R.sub.4 being .dbd.O, hydrogen, C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl, C.sub.(1-20) aminoalkyl, --(CH.sub.2).sub.q A(R.sub.5).sub.m, q being an integer from one to four, A being N or O, m being one or two and R.sub.5 being hydrogen, C.sub.(1-10) alkyl, C.sub.(1-10) alkoxyl, C.sub.(2-10) alkenyl, C.sub.(1-10) hydroxyalkyl, C.sub.(1-10) aminoalkyl, carbocyclic group or heterocyclic group;

R.sub.2 is a member selected from the group consisting of hydrogen, halogen, C.sub.(1-10) alkyl, C.sub.(1-10) alkoxyl, C.sub.(2-10) alkenyl, C.sub.(1-10) hydroxyalkyl, C.sub.(1-20) aminoalkyl, --A(R.sub.5).sub.m and --CHR.sub.6 A(R.sub.5).sub.m ; wherein A, R.sub.5 and m are as defined above, R.sub.6 is a C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl, C.sub.(1-20) aminoalkyl, carbocyclic group or heterocyclic group, or A is N, m is two and the two R.sub.5 join together with the A to form a heterocycle having from four to seven ring atoms selected from the group consisting of N, C and O, the A comprising a heteroatom of the heterocycle;

wherein at least one of: 1) R.sub.1 is NR.sub.3, O, or --CHR.sub.4 O-- or 2) R.sub.2 is --A(R.sub.5).sub.m ; and

wherein said carbocyclic group or heterocyclic group is a member selected from the group consisting of: anthracenyl, bicyclo[4.4.0]decanyl, bicyclo[2.2.1]heptanyl, bicyclo[3.2.0]heptanyl, bicyclo[4.1.0]heptanyl, bicylo[2.2.1]hexanyl, bicyclo[4.3.0]nonanyl, bicyclo[2.2.2]octanyl, biphenyl, cyclopentadienyl, cyclopentanyl, cyclobutanyl, cyclobutenyl, cycloheptanyl, cyclohexanyl, cyclooctanyl, cyclopropanyl, fluorenyl, indenyl, phenyl, quinonyl, napthalenyl, phenanthrenyl, azetidinyl, benzofuranyl, benzothiophenyl, carbazolyl, furanyl, glutarimidyl, indolyl, isoquinolinyl, oxazolyl, oxetanyl, oxiranyl, pyrrolidinyl, pyranyl, piperidinyl, pyridinyl, pyrrolyl, quinolinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydrothiophenyl and thiophenyl.

2. A compound selected from the group consisting of: ##STR9##

3. A pharmaceutical composition comprising a compound according to claim 1 and a suitable carrier, diluent or excipient.

4. The compound according to claim 1, wherein n is an integer from seven to sixteen.

5. The pharmaceutical composition of claim 3, wherein the composition is formulated for parenteral, topical or oral administration or for inhalation.

6. The compound according to claim 1, wherein j is one.

7. The compound according to claim 1, wherein R having formula I is attached to at least one of N.sub.1 or N.sub.3 xanthinyl nitrogens, the N.sub.7 xanthinyl nitrogen being substituted by a member selected from the group consisting of hydrogen, methyl or amino.

8. The compound according to claim 1, wherein R having formula I is bonded to an N.sub.1 nitrogen of xanthinyl, and wherein an N.sub.3 and an N.sub.7 xanthinyl nitrogens are independently substituted by a member selected from the group consisting of hydrogen, C.sub.(1-6) alkyl and amino.

9. A compound of the following formula:

10. A compound of the following formula:

11. A compound of the following formula:

12. A compound of the following formula:

13. A compound of the following formula:

14. A compound of the following formula:

Description

TECHNICAL FIELD OF THE INVENTION

The invention provides a group of compounds that are effective agents for inhibiting specific cellular signaling events often induced by inflammatory stimuli, or to be directly or indirectly antimicrobial to yeast or fungal infections. More specifically, the inventive compounds have at least one carboxylic acid, ester or amide-substituted chain bonded to a core moiety. The inventive compounds are, among other things, useful antagonists to control intracellular levels of specific sn-2 unsaturated phosphatidic acids and corresponding phosphatidic acid-derived diacylglycerols, intracellular cell signaling messengers which occur in response to pro-inflammatory proliferative stimuli.

BACKGROUND OF THE INVENTION

Pentoxifylline (1-(5-oxohexyl)-3,7-dimethylxanthine), abbreviated PTX, is a xanthine derivative which has seen widespread medical use for the increase of blood flow. PTX is disclosed in U.S. Pat. Nos. 3,422,107 and 3,737,433, both to Mohler et al. Metabolites of PTX were summarized in Davis et al., Applied Environment Microbiol. 48:327, 1984. A metabolite of PTX is 1-(5-hydroxyhexyl)-3,7-dimethylxanthine, designated M1. M1-was also disclosed as increasing cerebral blood flow in U.S. Pat. Nos. 4,515,795 and 4,576,947 to Hinze et al. Other metabolites, 1-(5-pentyl)-3,7-dimethylxanthine carboxylic acid, designated M5, and 1-(4-butyl)-3,7-dimethylxanthine carboxylic acid, designated M5, were disclosed by Bryce et al., Arzneim.-Forsch./Drug Res. 39(4):512-517, 1989. In addition, U.S. Pat. Nos. 4,833,146 and 5,039,666 to Gebert et al. and Novick, Jr., respectively, disclose use of tertiary alcohol analogs of xanthine for enhancing cerebral blood flow.

PTX and its known metabolites thereof have been shown to have in vivo activity in specific biologic systems. U.S. Pat. No. 4,636,507 to Kreutzer et al. describes an ability of PTX and M1, to further promote chemotaxis in polymorphonuclear leukocytes responding to a chemotaxis stimulator. In addition, PTX and related tertiary alcohol substituted xanthines inhibit activity of certain cytokines to affect chemotaxis (U.S. Pat. Nos. 4,965,271 and 5,096,906 to Mandell et al.). By administrating PTX and GM-CSF, patients undergoing allogeneic bone marrow transplant exhibited decreased levels of tumor necrosis factor, TNF, (Bianco et al., Blood 76: Supplement 1 (522A), 1990). Reduction in assayable levels of TNF was accompanied by a reduction in bone marrow transplant-related complications. However, in normal volunteers, TNF levels were higher

among PTX recipients. Therefore, elevated levels of TNF are not the primary cause of such complications.

Further research with PTX, its metabolites and their activity relating to various biologic systems spurred investigations with potential therapeutic agents heretofore unknown. These agents were identified as potential therapies for treating or preventing disease by inhibiting secondary cellular response to an external or in situ primary stimuli. These investigations sought to identify efficacious therapeutic compounds which were safe and effective for human or animal administration and maintain cellular homeostasis in the face of a variety of inflammatory stimuli.

In undertaking these investigations, previously unknown therapeutic compounds were discovered. These novel compounds are discussed herein. These compounds exhibit remarkable characteristics in predictive in vitro disease assays, which known compounds do not possess, indicating efficacious therapies for treating or preventing disease using the inventive compounds.

SUMMARY OF THE INVENTION

The invention provides carboxylic acid, ester and amide-substituted therapeutic compounds and pharmaceutical compositions and uses thereof. The inventive carboxylic acid, ester or amide-substituted compounds are useful in a large variety of therapeutic indications for treating or preventing disease. In particular, the inventive compounds and pharmaceutical compositions thereof provide therapy for diseases caused or advanced by intracellular signaling through specific intracellular signaling pathways, specifically the pathways discussed herein, by mediating a signaling response to an external stimuli. Abnormally-induced intracellular signaling is characteristic of diseases treatable using the inventive compounds or pharmaceutical compositions thereof.

The inventive compounds have at least one carboxylic acid, ester or amide-containing side chain and are preferably carbocyclic or heterocyclic compounds. The inventive compounds and pharmaceutical compositions thereof have the formula:

CORE MOIETY --(R).sub.j

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, wherein j is an integer from one to three, the core moiety is non-cyclic or cyclic (carbocyclic or heterocyclic) and R may be selected from among: hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, amino, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, carbocyclic or heterocyclic groups and formula I. The inventive compounds have at least one R of the following formula I: ##STR2## wherein: one or two p are the integer one, otherwise p is two;

n is an integer from three to twenty, preferably seven to sixteen, most preferably five to sixteen.

R.sub.1 can be selected from the group consisting of substituted and unsubstituted CH.sub.2 ; NR.sub.3, R.sub.3 being hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or C.sub.(1-20) hydroxyalkyl, or carbocyclic or heterocyclic group; O; --CHR.sub.4 O--, or --C(R.sub.4).sub.r O--, r being one or two, R.sub.4 being .dbd.O, hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl, C.sub.(1-20) aminoalkyl, --(CH.sub.2).sub.q A(R.sub.5).sub.m, q being an integer from one to four, A being N or O, m being one or two and R.sub.5 being hydrogen, a substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(1-10) alkoxyl, C.sub.(2-10) alkenyl or C.sub.(1-10) hydroxyalkyl, C.sub.(1-10) aminoalkyl, carbocyclic or heterocyclic group, or R.sub.2 and R.sub.4 join to form a substituted or unsubstituted heterocycle having four to seven ring atoms, the --O-- of --CHR.sub.4 O-- being a member of the heterocycle.

R.sub.2 can be selected from the group consisting of hydrogen; halogen; substituted or unsubstituted C.sub.(1-10) alkyl; C.sub.(1-10) alkoxyl; C.sub.(2-10) alkenyl; C.sub.(1-10) hydroxyalkyl, C.sub.(1-20) aminoalkyl; --A(R.sub.5).sub.m ; --CHR.sub.6 A(R.sub.5).sub.m ; A, R.sub.5 and m being defined above, R.sub.6 being a substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl, C.sub.(1-20) aminoalkyl, carbocyclic or heterocyclic groups, or A is N, m is two and the two R.sub.5 join to form a substituted or unsubstituted heterocycle having from four to seven ring atoms, A comprising a hetero atom of the heterocycle.

In the inventive compounds, at least one of R.sub.1 is NR.sub.3, O or --CHR.sub.4 O--, or R.sub.2 is --A(R.sub.5).sub.m. Optionally, (CH.sub.2).sub.n may 1) be substituted by a halogen, hydroxide, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, C.sub.(1-10) alkoxyl, C.sub.(1-10) acyloxy, C.sub.(1-

10) oxyalkyl, carbocyclic or heterocyclic group; 2) have one or two unsaturated bonds (preferably in a cis configuration); or 3) be interrupted by at least one oxygen atom.

A non-cyclic core moiety may include, but is not limited to, for example, acetamide, amide, amine, amino acid (one or two), carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol, thiolester or the like. Exemplary core moiety amino acids may include, but are not limited to, one or more of the following: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The non-cyclic core moiety may preferably be an amide, carboxyl ester, carboxide, hydrogen, hydroxide or a dipeptide comprising two amino acids selected from the foregoing exemplary list. A non-cyclic, halogen-core moiety may be, for example, bromine, chlorine, fluorine or iodine.

A cyclic core may be at least one five- to seven-member, non-heterocyclic ring (i.e., carbocycle) or a heterocycle. The at least one five- to seven-membered cyclic core may preferably have from one to three, five- to six-membered ring structures in a predominantly planar configuration. An exemplary, non-heterocyclic ring core moiety may be selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione; cyclopentanedione; napthlalene; phenol; quinone; salicylic acid; stilbene and tricyclododecane.

Although other heterocyclic cores are within the scope of the invention, the following representatives are preferred: substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic; quinolizinedione; quinazolinone; quinoline; recorsinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K; or xanthine.

Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimide, methylthymine, thymine, uracil or xanthine core. In representative, preferred compounds, R having formula I may be bonded to an N.sub.1 nitrogen of xanthine (and N.sub.3 and N.sub.7 xanthine nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, C.sub.(1-6) alkyl, fluoro, chloro and amino); or N.sub.1 nitrogen of uracil. Alternatively, R having formula I may be bonded to N.sub.1 and N.sub.3 xanthine nitrogens and the N.sub.7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino. Representative, preferred inventive compounds are compounds of formulas II, III and IV: ##STR3## wherein R is as defined above.

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier or diluent and some amount of an inventive compound. The nature of the composition and the pharmaceutical carrier or diluent will, of course, depend upon the intended route of administration, for example, parenterally, topically, orally or by inhalation for treatment of a patient with disease symptoms.

The invention also provides a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or in situ primary stimuli. Treatment of the disease states involves mediating the cellular response through a specific phospholipid-based second messenger acting adjacent to a cell membrane inner leaflet. The second messenger pathway is activated in response to various noxious or proliferative stimuli, characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. Biochemistry of this second messenger pathway is described herein. More specifically, the invention includes methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid and through glycan phosphatidylinositol (Gly PI).

Gly PI consists of a phosphatidylinositol-1-phosphate (PIP) bound through the carbon 6-hydroxyl to a glucosamine residue, which in turn is bound, usually to 2-5 other glycan residues (1.fwdarw.4 type, linear bonds) containing an additional one to three phosphoethanolamine moieties, the last of which may be bound to an external protein such as Thy-1. Evidence suggests a broad variety of structural variation in the sn-1 and sn-2 positions of the glycerol/lipid moiety of the phosphatidylinositol, as well as fatty acyl addition to the 2-OH group of the inositol. Several functional parameters of structure have been observed, the most remarkable of which point to a minimum presence of at least one myristoyl sidechain in Gly-PI molecules, the presence of both alkyl (ether) and acyl chains in the sn-l position, and the presence of palmitate (C16:0) in the 2-OH

position of the inositol in protein-binding Gly-PI. Thomas et al., Biochemistry 29: 5413-5422 (1991).

Recent research has demonstrated that 2-OH-acylation of the inositol moiety conveys resistance to hydrolysis with Gly PI-directed phospholipase C (P.sub.i G-PLC, a phosphodiesterase which hydrolyzes Gly PI to glycan inositol phosphate and diacylglycerol) but not to Gly PI-directed phospholipase D (P.sub.i G-PLD, a phosphodiesterase which hydrolyzes Gly PI to glycan inositol+phosphatidic acid).

Research has identified two functions of Gly-PI: 1) external protein binding, the purpose of which may be simple binding to the cell membrane or placement of conformational constraints on the structure of externally bound membrane proteins (e.g., so that a particular portion of the molecule faces an extracellular environment); and 2) signal transduction, including part of the intracellular signal sent by insulin and a detectable portion of the signal transduced by Interleukin-2 (IL-2). We have found that signal transducing Gly-PI in B lymphocytes is hydrolyzed following anti-mu crosslinking, and then resynthesized rapidly. In these systems, two Gly-PI species are synthesized: a) GlyPI.sub.1, containing 1-myristoyl 2-palmitoyl, 1-o-tetradecanyl (myristyl) 2-palmitoyl and 1-myristyl 2-myristyl phosphatidylinositol; and b) Gly PI.sub.2, containing 1-myristoyl 2-oleoyl and 1-o-myristyl 2-linoleoyl phosphatidylinositol. Fraction (a) above contains a 1:1 mole content of C22 or C20 acyl groups attached to the inositol phosphate. The Gly-PI.sub.1 fraction, identified by glucosamine labeling followed by mass spectrometry, exhibits a characteristic tripartite peak (glycan-inositol: 2-OH-acyl: phosphatidic acid moieties) and is uniformly inositol 2-OH acylated. Therefore, fraction (a) conveys resistance to P.sub.i G-PLC but not to P.sub.i G-PLD, suggesting that the observed fraction, when hydrolyzed, will generate 1-myristyl and 1-o-myristyl phosphatidic acid species, subsequently observed.

Thus, inventive compounds, useful in treating diseases and reducing toxicity of other disease treatments, would affect cellular signaling through a second messenger pathway by interacting with binding and/or signaling functions of Gly PI.

A disease state or treatment-induced toxicity are selected from the group consisting of: tumor progression involving tumor stimulation of blood supply (angiogenesis) by production of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF); tumor invasion and formation of metastases through adhesion molecule binding, expressed by vascular endothelial cells (VCAM and ICAM); tissue invasion through tumor metalloprotease production such as MMP-9; autoimmune diseases caused by dysregulation of the T cell or B cell immune systems, treatable by suppression of the T cell or B cell responses; acute allergic reactions including, but not limited to, asthma and chronic inflammatory diseases, mediated by pro-inflammatory cytokines including tumor necrosis factor (TNF) and IL-1, and rheumatoid arthritis, osteoarthritis, multiple sclerosis or insulin dependent diabetes mellitus (IDDM), associated with enhanced localization of inflammatory cells and release of inflammatory cytokines and metalloproteases; smooth muscle cell, endothelial cell, fibroblast and other cell type proliferation in response to growth factors, such as PDGF-AA, BB, FGF, EGF, etc. (i.e., atherosclerosis, restenosis, stroke, and coronary artery disease); activation of human immunodeficiency virus infection (AIDS and AIDS related complex); HIV-associated dementia; kidney mesangial cell proliferation in response to IL-1, MIP-1.alpha., PDGF or FGF; inflammation; kidney glomerular or tubular toxicity in response to cyclosporin A or amphotericin B treatment; organ toxicity (e.g., gastrointestinal or pulmonary epithelial) in response to a cytotoxic therapy (e.g., cytotoxic drug or radiation); effects of non-alkylating anti-tumor agents; inflammation in response to inflammatory stimuli (e.g., TNF, IL-1 and the like) characterized by production of metalloproteases or allergies due to degranulation of mast cells and basophils in response to IgE or RANTES; bone diseases caused by overproduction of osteoclast-activating factor (OAF) by osteoclasts; CNS diseases resulting from over-stimulation by pro-inflammatory neurotransmitters such as, acetylcholine, serotonin, leuenkephalin or glutamate; acute inflammatory diseases such as septic shock, adult respiratory distress syndrome; multi-organ dysfunction associated with inflammatory cytokine cascade; and combinations thereof.

In a large number of cells, signaling is dependent upon generation of a broad variety of PA species, some of which are generated from lyso-PA by the enzyme lyso-PA acyl transferase and some of which are generated from 2--O-- acyl glycan-PI by P.sub.i G-PLD. Generation of each of these PA species (the predominant forms being: 1-acyl and 1-alkyl 2-linoleoyl PA compounds, generated by LPAAT; and 1-myristyl 2-palmitoyl and 1-o-myristyl 2-palmitoyl, generated by P.sub.i G-PLD) serves to effect both proliferative and/or inflammatory signaling in the diseases discussed and cell systems described above.

The inventive compounds are of particular significance for inhibiting IL-2-induced proliferative response. IL-2 signaling inhibition is potentially useful in the treatment of numerous disease states involving T-cell activation and hyperproliferation. Exemplary autoimmune diseases treated by inhibiting IL-2 signaling are lupus, scleroderma, rheumatoid arthritis, multiple sclerosis, glomerula nephritis as well as potential malignancies,

including but not limited to, chronic myelogenous leukemia as well as others.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1 and 2 are dose response curves prepared from results in a murine thymocyte assay, determining inhibitive effects of inventive compounds nos. 3546 and 3549, (see below for chemical name and structure) respectively, on proliferation of thymocytes co-stimulated by ConA and IL-2.

FIGS. 3 and 4 are plotted graphs of compound concentrations (.mu.M) versus inhibition (as a function of incorporated thymidine, cpm) for compounds nos. 1514 and 1583, respectively, in a mixed lymphocyte reaction (MLR) assay.

FIG. 5 reports the experimentally calculated IC50 values obtained in the an assay investigating inhibitive effects of various inventive compounds on proliferation of Balb/3T3 cells in response to stimulation by PDGF. In addition, FIG. 5 reports LD50 values for each inventive compound tested in the proliferation assay. The reported LD50 values were obtained in a corresponding viability assay.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The invention provides a genus of compounds which can control cellular behavior by a particular phase of a secondary messenger pathway system (Bursten et al., J Biol. Chem. 266:20732, 1991). The second messengers are lipids or phospholipids and use the following abbreviations:

PE=phosphatidyl ethanolamine

LPE=lysophosphoethanolamine

PA=phosphatidic acid

LPA=lysophosphatidic acid

DAG=diacylglycerol

LPLD=lysophospholipase-D

LPAAT=lysophosphatidic acid acyl transferase

PAPH=phosphatidic acid phosphohydrolase

PLA2=phospholipase A2.

PLD=phospholipase D

PAA=phosphoarachidonic acid

PC=phosphatidyl choline

"remodeled" PA, cyclic pathway=PAA, LPA, PA and DAG intermediates substituted with 1-saturated, 2-linoleoyl or 1,2-dioleoyl, dioleoyl/1,2-sn-dilinoleoyl at the indicated sn-1 and sn-2 positions.

"Classical PI Pathway"=PI, DAG, PA intermediates substituted with 1-stearoyl, 2-arachidonoyl fatty acyl side chains.

"PLD-generated PA"=PE, PC, LPA, PA and DAG intermediates substituted with, e.g., 1,2-sn-dioleoyl-, 1-alkyl, 2-linoleoyl-, and 1-alkyl, 2-docosahexaenoyl-side chains.

Lysophosphatidic acid transferase (LPAAT) effects the synthesis of phosphatidic acid (PA) from lysophosphatidic acid (LPA) by incorporation of an acyl group from acyl CoA. Hydrolysis of the phosphate moiety by PA phosphohydrolase (PAPH) results in the formation of DAG. These aspects of the pathway appear to be activated immediately (within a minute) upon stimulation by a primary stimulus (e.g., a cytokine such as IL-1, IL-2 or TNF) acting at a receptor on a cellular surface. An immediate detectable effect is an elevation of levels of PA and DAG. Administration of the compounds of the invention reverse this elevation.

The compounds and pharmaceutical compositions of the invention include, but are not limited to, inhibitors of subspecies of LPAAT and PAPH enzymes with substrate specificity for intermediates with 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. One representative example of such an inhibitor (although not within the genus of inventive compounds) is PTX. PTX blocks PAPH in a specific activation pathway that does not involve PI but rather derives from a PA that is largely composed of 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. This was shown, for example, by the demonstration that human mesangial cells stimulated with TNF produce DAG from PI and regenerate PI in the absence and the presence of PTX. In the latter system there is no evidence to suggest that PA or DAG are derived from sources other than PI. It should be emphasized that the compounds of the invention affect that subset of PAPH and LPAAT that relates to substrates with unsaturated fatty acids other than arachidonate in the sn-2 position, not the housekeeping forms of these enzymes that serve the PI pathway.

Each membrane phospholipid subclass (e.g., PA, PI, PE, PC and PS) reaches a stable content of characteristic fatty acyl side chains due to cyclic remodeling of the plasma membrane as well as turnover for each subclass. PA is often stable, but present in relatively small quantities. PA in resting cells consists mostly of saturated acyl chains, usually consisting of myristate, stearate and palmitate. In resting cells, PC's acyl side chains consist mostly of acyl palmitate in the sn-1 position and oleate in the sn-2 position. PE and PI are predominantly composed of sn-1 stearate and sn-2 arachidonate.

Due to this characteristic content of acyl groups in the sn-1 and sn-2 positions, the origin of any PA species may be deduced from the chemical nature of its acyl groups in the sn-1 and sn-2 positions. For example, if PA is derived from PC through action of the enzyme PLD, the PA will contain the characteristic acyl side chains of PC substrate passed through the second messenger pathway. Further, the origin of any 1,2 sn-substrate species may be differentiated as to its origin. It is important to know whether or not each phospholipid species passes through a PA form prior to hydrolysis to DAG. The lyso-PA that is converted to PA and then to DAG may be shown. The complexities of this second messenger pathway can be sorted by suitable analyses using fatty acyl side chain chemistry (e.g., by thin layer chromatography, gas-liquid chromatography, or high pressure liquid chromatography) of intermediates in cells at various time points after stimulation of the second messenger pathway.

In certain mesenchymal cells, such as neutrophils and rat or human mesangial cells, several signaling pathways may be activated in tandem, simultaneously or both. For example, in neutrophils, F-Met-Leu-Phe stimulates formation of PA through the action of PLD, followed in time by formation of DAG through PAPH action. Several minutes later, DAG is generated from PI through the classical phosphoinositide pathway. In many cells, DAG is derived from both PA that is remodeled through a cycle whereby PA is sn-2 hydrolyzed by PLA2, followed by sn-2 transacylation by LPAAT and PA that is generated in a PLD-pathway from either PE or PC or both substrates by PLD.

The present second messenger pathway involves substrates with unsaturated fatty acids in the sn-2 position other than arachidonate and those sub-species of PAPH and LPAAT that are not involved in normal cellular housekeeping functions that are part of the classical PI pathway. The PAPH and LPAAT enzymes involved in this specific second messenger pathway are exquisitely stereo-specific for different acyl side chains and isomeric forms of substrates. Therefore, the inventive compounds may preferably be substantially enantiomerically pure.

PTX (in vitro) blocks formation of remodeled PA through the PA/DAG pathway at high PTX concentrations (greater than those that could be achieved in patients without dose-limiting side effects) by blocking formation of PA subspecies at LPAAT. Even in the presence of PTX, cells continue to form PA through the action of PLD, and DAG is also formed through the action of phospholipase C on PC and PI. The latter pathway are not inhibited by the inventive compounds or PTX. In PTX-treated cells, DAG derived from remodeled and PLA-generated PA is diminished (e.g., 1,2-sn-dioleoyl DAG, 1-alkyl, 2-linoleoyl DAG and 1-alkyl, 2-docosahexaneolyl DAG). Therefore, the inventive compounds and PTX inhibit the formation of only a certain species of PA and DAG by selectively inhibiting a specific second messenger pathway that is only activated in cells by noxious stimuli, but is not used to signal normal cellular housekeeping functions.

Therapeutic Uses of the Inventive Compounds

The specific activation inhibition of the second messenger pathway, as described above and activated primarily by various noxious stimuli, suggests that the inventive compounds are useful in treating a wide variety of clinical indications, mediated at the cellular level by a common mechanism of action. Moreover, in vitro and in vivo data presented herein provides predictive data that a wide variety of clinical indications,

having similar effects on the specific second messenger pathway (activated by noxious stimuli and mediated through, for example, inflammatory cytokines), may be treated by the inventive compounds, which specifically inhibit the pathway. In fact, the mechanism of action for the inventive compounds explains why these compounds have multifarious clinical indications.

Activation of the second messenger pathway is a major mediator of response to noxious stimuli and results in cellular signals that lead to, for example, acute and chronic inflammation, immune response and cancer cell growth. Although the inventive compounds may desirably inhibit other noxious stimuli not discussed, they most effectively mediate the above conditions. Signals mediated by the present second messenger pathway include, for example, those cellular responses of LPS directly; T cell activation by antigen; B cell activation by antigen, cellular responses to IL-1, mediated through the IL-1 Type I receptor (but not the IL-1 Type II receptor), and TNF (Type I receptor), growth stimulated by transformations including, but not limited to, activated oncogenes (e.g., ras, abl, her 2-neu and the like), smooth muscle cell proliferation stimulated by PDGF, b-FGF and IL-1; T cell and B cell growth stimulation by IL-2, IL-4 or IL-7 and IL-4 or IL-6, respectively; and more generally, T cell receptor signaling.

In vitro, the inventive compounds: (1) block IL-1 signal transduction through the Type 1 receptor as shown, for example, by preventing IL-1 and IL-1 plus PDGF (platelet derived growth factor) induction of proliferation of smooth muscle, endothelial and kidney mesengial cells; (2) suppress up-regulation of adhesion molecules as shown, for example, by blocking VCAM in endothelial cells; (3) inhibit TNF, LPS and IL-1 induced metalloproteases (an inflammation model); (4) block LPS, TNF or IL-1 induced metalloprotease and secondary cytokine production (for prevention and treatment of septic shock); (5) suppress T cell and B cell activation by antigen, for example, IL-2 and IL-4; (6) inhibit mast cell activation by IgE; (7) are cytotoxic for transformed cells and tumor cell lines, yet not for normal cells; and (8) block signaling by IL-2, IL-4, IL-6 and IL-7 on T and B cells.

The foregoing in vitro effects give rise to the following in vivo biological effects, including, but not limited to: protection and treatment of endotoxic shock and sepsis induced by gram positive or gram negative bacteria; inhibition of tumor cell growth; synergistic immunosuppression, active in autoimmune diseases and in suppressing allograft reactions; and stimulation of hair grow through reversal of an apoptotic process. The inventive compounds are most potent when used to prevent and treat septic shock, treat acute and chronic inflammatory disease, treat or prevent an autoimmune disease and stimulate hair growth (when applied topically).

The inventive compounds also are useful as an adjuvant to inhibit toxic side effects of drugs whose side effects are mediated through the present second messenger pathway.

Metalloproteases mediate tissue damage such as glomerular diseases of the kidney, joint destruction in arthritis, and lung destruction in emphysema, and play a role in tumor metastases. Three examples of metalloproteases include a 92 kD type V gelatinase induced by TNF, IL-1 and PDGF plus bFGF, a 72 Kd type IV collagenase that is usually constitutive and induced by TNF or IL-1, and a stromelysin/PUMP-1 induced by TNF and IL-1. The inventive compounds can inhibit TNF or IL-1 induction of the 92 kD type V gelatinase inducable metalloprotease. Moreover, the inventive compounds can reduce PUMP-1 activity induced by 100 U/ml of IL-1. Accordingly, the inventive compounds prevent induction of certain metalloproteases induced by IL-1 or TNF and are not involved with constitutively produced proteases (e.g., 72 kD type IV collagenase) involved in normal tissue remodeling.

The inventive compounds inhibit signal transduction mediated through the Type I IL-1 receptor, and are therefore considered as IL-1 antagonists. A recent review article entitled "The Role of Interleukin-1 in Disease" (Dinarello et al., N. Engl. J. Med. 328, 106, Jan. 14, 1993) described the role of IL-1 as "an important rapid and direct determinant of disease . . . In septic shock, for example, IL-1 acts directly on the blood vessels to induce vasodilatation through the rapid production of platelet activating factor and nitric oxide, whereas in autoimmune disease it acts by stimulating other cells to produce cytokines or enzymes that then act on the target tissue." The article describes a group of diseases that are mediated by IL-1, including sepsis syndrome, rheumatoid arthritis, inflammatory bowel disease, acute and myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis and other diseases including transplant rejection, graft versus host disease (GVHD), psoriasis, asthma, osteoporosis, periodontal disease, autoimmune thyroiditis, alcoholic hepatitis, premature labor secondary to uterine infection and even sleep disorders. Since the inventive compounds inhibit cellular signaling through the IL-1 Type I receptor and are IL-1 antagonists, the inventive compounds are useful for treating all of the above-mentioned diseases.

For example, for sepsis syndrome, the mechanism of IL-1-induced shock appears to be the ability of IL-1 to

increase the plasma concentrations of small mediator molecules such as platelet activating factor, prostaglandin and nitric oxide. These substances are potent vasodilators and induce shock in laboratory animals. Blocking the action of IL-1 prevents the synthesis and release of these mediators. In animals, a single intravenous injection of IL-1 decreases mean arterial pressure, lowers systemic vascular resistance, and induces leukopenia and thrombocytopenia. In humans, the intravenous administration of IL-1 also rapidly decreases blood pressure and doses of 300 ng or more per kilogram of body weight may cause severe hypotension. The therapeutic advantage of blocking the action of IL-1 resides in preventing its deleterious biological effects without interfering with the production of molecules that have a role in homeostasis. The present inventive compounds address this need, identified by Dinarello et al., by inhibiting cellular signaling only through the IL-1 Type I receptor and not through the IL-1 Type II receptor.

With regard to rheumatoid arthritis, Dinarello and Wolff state: "Interleukin-1 is present in synovial lining and synovial fluid of patients with rheumatoid arthritis, and explants of synovial tissue from such patients produce IL-1 in vitro. Intraarticular injections of interleukin-1 induce leukocyte infiltration, cartilage breakdown, and periarticular bone remodeling in animals. In isolated cartilage and bone cells in vitro, interleukin-1 triggers the expression of genes for collagenases as well as phospholipases and cyclooxygenase, and blocking its action reduces bacterial-cell-wall-induced arthritis in rats." Therefore, the inventive compounds, as IL-1 antagonists, are useful to treat and prevent rheumatoid arthritis.

With regard to inflammatory bowel disease, ulcerative colitis and Crohn's disease are characterized by infiltrative lesions of the bowel that contain activated neutrophils and macrophages. IL-1 can stimulate production of inflammatory eicosanoids such as prostaglandin E.sub.2 (PGE.sub.2), leukotriene B.sub.4 (LTB.sub.4) and IL-8, an inflammatory cytokine with neutrophil-chemoattractant and neutrophil-stimulating properties. Tissue concentrations of PGE2 and LTB4 correlate to severity of disease in patients with ulcerative colitis, patients with inflammatory bowel disease having high tissue concentrations of IL-1 and IL-8. Therefore, an IL-1 antagonist, such as the inventive compounds, would be effective to treat inflammatory bowel disease.

With regard to acute and chronic myelogenous leukemia, there is increasing evidence that IL-1 acts as a growth factor for such tumor cells. Therefore, the inventive compounds should be effective to prevent the growth of worsening of disease for acute and chronic myelogenous leukemias.

Insulin-dependent diabetes mellitus (IDDM) is considered to be an autoimmune disease with destruction of beta cells in the islets of Langerhans, mediated by immunocompetent cells. Islets of animals with spontaneously occurring IDDM (e.g., BB rats or NOD mice) have inflammatory cells that contain IL-1. Therefore, the inventive compounds should be useful for the preventing and treating IDDM.

IL-1 also plays a role in atherosclerosis development. Endothelial cells are a target of IL-1. IL-1 stimulates proliferation of vascular smooth muscle cells. Foam cells, isolated from fatty arterial plaques from hypercholesterolemic rabbits, contain IL-1.beta. and IL-1.beta. messenger RNA. The uptake of peripheral blood monocytes results in initiation of IL-1 production by these cells. IL-1 also stimulates production of PDGF. Taken together, IL-1 plays a part in the development of atherosclerotic lesions. Therefore, an IL-1 antagonist, such as the inventive compounds should be useful in preventing and treating atherosclerosis.

IL-1 activates (through the Type I IL-1 receptor) a lyso-PA acyltransferase (LPAAT) and phosphatidate phosphohydrolase within 5 seconds of cell (for example, human mesangial cells, HMC) exposure to this cytokine. As discussed in detail above, activation of both enzymes results in production of PA species with sn-1 and sn-2 unsaturated acyl groups, with the majority of sn-2 acyl chains being polyunsaturated. Both IL-1 and a product of LPAAT, 1,2-sn-dilinoleoyl PA, activate a signaling pathway involving hydrolysis of PE to PA. This reaction is followed by dephosphorylation of PA to produce both 1,2-sn-diacylglycerol, and 1-o-alkyl, or 1-o-alkenyl,acylglycerol (AAG) species. The inventive compounds exert their activity by inhibiting one or both enzymes at an inner leaflet of the plasma membrane. Therefore, appropriate in vitro models for drug activity may measure inhibition of stimulation caused by a proinflammatory cytokine or other inflammatory cellular signal.

The generation of the sn-2 unsaturated PA fraction by LPAAT serves to activate either G-proteins, or acts directly upon PLD through alteration of its lipid microenvironment. Activation of LPAAT and generation of the sn-2-unsaturated PA species is an energy sensitive pathway of PLD. This provides a mechanism for a limited-receptor system to amplify a signal and generate a cellular response by rapid synthesis of small amounts of PA. Uptake of di-unsaturated PA, which is less than about 0.1% of total membrane lipid mass, is sufficient to activate PLD activity. This quantity of PA is similar to that endogenously synthesized by LPAAT. The PA-stimulated PLD acts upon PE, which should be localized to the inner leaflet of the cell membrane, enriched in PE relative to the outer leaflet. Therefore, the cellular inflammatory response to IL-1 is mediated by the

pathway: IL-1R.fwdarw.PA.fwdarw.(PLD).fwdarw.PE. Whereas a localized tissue response is: lysoPA.fwdarw.PI.fwdarw.PKC.fwdarw.(PLD).fwdarw.PC. The PLD species are likely to be different isozymes. The second messenger pathway whose activation is inhibited by the inventive compounds is not a PI-derived pathway and does not involve PKC in the time courses of inhibition. PKC is acutely activated by PI-derived DAG, but chronic activation (ie.,>30 minutes) is maintained by PC-derived PA generated by PC-directed PLD. Therefore, the pathway inhibited by the inventive compounds is PE-directed and not PC-directed. Moreover, the PE-directed PLD favors substrates with sn-2 long-chain unsaturation.

DAG and PA are upregulated in oncogenically transformed cells. For example, activating ras mutations result in increased generation of DAG upon stimulation with mitogens, although the sources of DAG differ between experimental systems. In nontransformed renal mesangial cells, IL-1.beta. stimulation increased PLA2 and LPAAT activation, resulting in generation of sn-2 unsaturated PA and subsequent hydrolysis to DAG by phosphatidate phosphohydrolase. The ras transformation in NIH/3T3 cells upregulates serum-stimulated generation of DAG and PA. Particular species of DAG that is stimulated by serum is dioleoyl and of PA are dilinoleoyl and dioleoyl. This upregulation occurs over 4-12 hours and pretreatment of cells with an inventive compound, or PTX, blocks generation of these phospholipid second messengers. The inhibition occurs either through suppressing the generation of PA de novo from lysoPA, or through inhibition of one or both arms of the Lands cycle. The coordinate increase of lysoPA in the setting of diminished PA/DAG production suggests inhibition of transacylation of a precursor lipid. Therefore, the ras transformation mediates an upregulation of PA through indirect stimulation of PLA2 and/or LPAAT activity. The inventive compounds inhibit the conversion of the upregulated lysoPA to PA and subsequently block the phenotypic changes induced by PA/DAG in the membrane.

The ability of the inventive compounds to inhibit generation of unsaturated phospholipids is mirrored by the ability of inventive compounds to inhibit proliferation and tumorigenicity of ras-transformed cells in vitro and in vivo. PTX inhibits ras-transformed NIH3T3 cells more than parental cells. This inhibition is reversible and is not associated with significant cytotoxicity.

Excessive or unregulated TNF (tumor necrosis factor) production is implicated in mediating or exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft versus host reaction, allograft rejections, fever, myalgias due to infection such as influenza, cachexia secondary to infection, AIDS or malignancy, AIDS, other viral infections (e.g., CMV, influenza, adenovirus, herpes family), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, or pyresis. The inventive compounds or pharmaceutically acceptable salts thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human or other mammal, which is exacerbated or signaled through the present second messenger cellular phospholipid-based signaling pathway and by excessive or unregulated production of "first messenger" inflammatory cytokines such as TNF or IL-1. With regard to TNF first messenger signaling, there are several disease states in which excessive or unregulated TNF production by monocytes/macrophages is implicated in exacerbating or causing the disease. These include, but are not limited to, for example, neurodegenerative diseases such as Alzheimers disease, endotoxemia or toxic shock syndrome (Tracey et al., Nature 330:662, 1987 and Hinshaw et al., Circ. Shock 30:279, 1990); cachexia (Dezube et al., Lancet 355:662, 1990), and adult respiratory distress syndrome (Miller et al., Lancet 2(8665):712, 1989). The inventive compounds may be used topically in the treatment of prophylaxis of topical disease states mediated or exacerbated by excessive TNF or IL-1, such as viral infections (herpes or viral conjunctivitis), psoriasis, fungal or yeast infections (ringworm, athletes foot, vaginitis, dandruff, etc.) or other dermatologic hyperproliferative disorders. High TNF levels have been implicated in acute malaria attacks (Grau et al., N. Engl. J Med. 320:1585, 1989), chronic pulmonary inflammatory diseases such as silicosis and asbestosis (Piguet et al., Nature 344:245, 1990, and Bissonnette et al., Inflammation 13:329, 1989), and reperfusion injury (Vedder et al., Proc. Natl. Acad. Sci. USA 87:2643, 1990).

The compounds of the invention can inhibit certain VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor) and PDGF (platelet derived growth factor) effects in vivo, such as inhibition of angiogenesis or restenosis. For example, Ferns et al., Science 253:1129, 1991, have shown that neointimal smooth muscle chemotaxis and angioplasty are inhibited in rats using a neutralizing antibody to PDGF. Also, Jawien et al., J. Clin Invest. 89:507, 1992, have shown that PDGF promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. Inhibition of the PDGF-mediated effects following balloon angioplasty by the inventive compounds is the pharmacological rationale for using the inventive compounds as therapeutic agents to prevent restenosis. The inventive compounds also inhibit atherogenesis because increased levels of

PDGF expressed by macrophages are associated with all phases of atherogenesis (Ross et al., Science 248:1009, 1990). Further, many human tumors express elevated levels of either PDGF, FGF, receptors for FGF or PDGF, or mutated cellular oncogenes highly homologous to these growth factors or their receptors. For example, such tumor cell lines include sarcoma cell lines (Leveen et al., Int. J. Cancer 46:1066, 1990), metastatic melanoma cells (Yamanishi et al., Cancer Res. 52:5024, 1992), and glial tumors (Fleming et al., Cancer Res. 52:4550, 1992).

The inventive compounds are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and generally improve memory in subjects with organic deficits, including Alzheimer's patients.

Compounds of the Invention

The invention provides compounds that are useful therapeutic agents, inhibiting proinflammatory and neoplastic cellular signalling mechanisms. The inventive compounds and inventive pharmaceutical compositions thereof have the formula:

CORE MOIETY --(R).sub.j

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, wherein j is an integer from one to three, the core moiety is non-cyclic or cyclic (e.g. carbocyclic or heterocyclic) and R may be selected from among: hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, amino, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, carbocyclic or heterocyclic groups and formula I.

Preferred R substituents having a structure other than formula I include, but are not limited to, 2-bromopropyl, 4-chloropentyl, cyclohexyl, cyclopentyl, 3-dimethylaminobutyl, ethyl, hexyl, 2-hydroxyethyl, 5-hydroxyhexyl, 3-hydroxy-n-butyl, 3-hydroxypropyl, isobutyl, isopropyl, 2-methoxyethyl, 4-methoxy-n-butyl, methyl, n-butyl, n-propyl, phenyl, t-butyl and the like. Particularly preferred R having a structure other than formula I are ethyl, methyl, or hydrogen.

The inventive compounds have at least one R of the following formula I: ##STR4## wherein: one or two p are the integer one, otherwise p is two;

n is an integer from three to twenty.

R.sub.1 is selected from among substituted and unsubstituted CH.sub.2 ; NR.sub.3 (R.sub.3 being hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or C.sub.(1-20) hydroxyalkyl, or carbocyclic or heterocyclic group); O; --CHR.sub.4 O-- (R.sub.4 being substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, C.sub.(1-20) hydroxyalkyl or R.sub.2 and R.sub.4 join to form a substituted or unsubstituted heterocycle having four to seven ring atoms, the ether group --O-- of --CHR.sub.4 O-- being a member of the heterocycle); R.sub.2 is hydrogen, halogen, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(1-10) alkoxyl, C.sub.(2-10) alkenyl, C.sub.(1-10) hydroxyalkyl, --A(R.sub.5).sub.m (A being N or O, m being one or two and R.sub.5 being hydrogen, a substituted or unsubstituted carbocyclic or heterocyclic group having at least one four- to seven-membered ring; substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(1-10) alkoxyl, C.sub.(2-10) alkenyl or C.sub.(1-10) hydroxyalkyl).

In the inventive compounds, at least one of R.sub.1 is NR.sub.3, O or --CHR.sub.4 O--, or R.sub.2 is --A(R.sub.5).sub.m. In addition when the core moiety is xanthine, R.sub.2 is --A(R.sub.5).sub.m, A is --O-- and R.sub.5 is hydrogen or C.sub.(1-10) alkyl, n is not less than four. Optionally, (CH.sub.2).sub.n may 1) be substituted by a halogen, hydroxide, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, C.sub.(1-10) alkoxyl, C.sub.(1-10) acyloxy, C.sub.(1-10) oxyalkyl, carbocyclic or heterocyclic group; 2) have one or two unsaturated bonds (preferably in a cis configuration); or 3) be interrupted by at least one oxygen atom.

Preferably, n is an integer from about three to about eighteen, more preferably, an integer from about four to about ten. In especially preferred compounds, R.sub.1 is NR.sub.3, R.sub.3 is C.sub.(1-20) alkyl, and R.sub.2 is C.sub.(1-10) alkyl or hydroxyalkyl. Even more preferably, (CH.sub.2).sub.n is substituted by an hydroxide, a C.sub.(1-10) alkyl or C.sub.(1-10) acyloxy. Other preferred embodiments may include, but are not limited to, compounds in which R.sub.1 is O, R.sub.2 is C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl or C.sub.(1-10) alkoxyl and (CH.sub.2).sub.n is substituted by a halo-substituted C.sub.(1-10) alkyl, or unsubstituted C.sub.(2-10) alkenyl or C.sub.(1-10) alkoxyl.

Although other possible substituents are within the scope of the inventive compounds, representative substituents, when R, R.sub.2 or R.sub.5 is a substituted C.sub.(1-10) alkyl, C.sub.(2-10) alkoxyl, C.sub.(2-10) alkenyl or C.sub.(1-10) hydroxyalkyl, may be: amide, primary, secondary and tertiary amine, C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl (including, e.g., branched and unbranched alkyl or alkenyl groups), C.sub.(1-8) alkoxyl, C.sub.(1-8) hydroxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, C.sub.(1-8) haloalkyl (including, e.g., mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanate, isothiocyanate, phosphate, phosphonate, primary, secondary or tertiary alcohol (including, e.g., any one of various diols, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol, and methylcyclohexanol), sulfonate, sulfone, sulfoxide, thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea.

The above-listed, substituents are also representative of substituents when R.sub.3 or R.sub.4 is a substituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or C.sub.(1-20) hydroxyalkyl; R, R.sub.3 or R.sub.5 is a substituted carbocyclic or heterocyclic group; or R.sub.1 is a substituted CH.sub.2.

Representative R, R.sub.3 or R.sub.5 carbocyclic or heterocyclic groups may be, but are not limited to: anthracene, bicyclo[4.4.0]decane, bicyclo[2.2.1]heptane, bicyclo[3.2.0]heptane, bicyclo[4.1.0]heptane, bicyclo[2.2.1]hexane, bicyclo[4.3.0]nonane, bicyclo[2.2.2]octane, biphenyl, cyclopentadiene, cyclopentane, cyclobutane, cyclobutene, cycloheptane, cyclohexane, cyclooctane and cyclopropane, 1,2-diphenylethane, fluorene, indene, phenyl, quinone, terphenyl, napthalene, phenanthrene, terphenyl, toluene, xylene, azetidine, benzofuran, benzothiophene, carbazole, furan, glutarimide, indole, isoquinoline, lactam, lactone, oxazole, oxetane, oxirane, phthalimide, piperidine, pyrrolidine, pyran, pyridine, pyrrole, quinoline, tetrahydrofuran, tetrahydropyran, tetrahydrothiophene, thiophene, thymine, derivatives thereof and the like. Due primarily to availability and ease of synthesis, more preferred cyclic (carbocyclic or heterocyclic) groups include, but are not limited to, less complex ring systems, such as, for example, cyclopentane and cyclohexane, cyclopentadiene, phenyl, indene, toluene, xylene, furan, indole, thymine and xanthine.

A non-cyclic core moiety may include, but is not limited to, for example, acetamide, amide, amine, amino acid (one or two), carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol, thiolester or the like. Exemplary core moiety amino acids may include, but is limited to, one or more of the following: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The non-cyclic core moiety may preferably be an amide, carboxyl ester, carboxide, hydrogen, hydroxide or a dipeptide comprising two amino acids selected from the foregoing exemplary list. A non-cyclic, halogen-core moiety may be, for example, bromine, chlorine, fluorine or iodine.

A cyclic core may be at least one five- to seven-member, non-heterocyclic (i.e. carbocyclic) ring or a heterocycle. The at least one five- to seven-membered cyclic core may preferably have from one to three, five- to six-membered ring structures in a predominantly planar configuration. An exemplary, non-heterocyclic ring core moiety may be selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione; cyclopentanedione; napthlalene; phenol; quinone; salicylic acid; stilbene and tricyclododecane.

Although other heterocyclic cores are within the scope of the invention, the following representatives are preferred: substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic; quinolizinedione; quinazolinone; quinoline; recorsinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K; or xanthine.

Representative substituents for the non-heterocyclic (i.e., carbocyclic) or heterocyclic core moieties include, for example, amide, primary, secondary and tertiary amine, C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl (including,

e.g., branched and unbranched alkyl or alkenyl groups), C.sub.(1-8) alkoxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, C.sub.(1-8) haloalkyl (including, e.g., mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanate, isothiocyanate, phosphate, phosphonate, primary, secondary or tertiary alcohol (including, e.g., any one of various diols, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol, and methylcyclohexanol), sulfonate, sulfone, sulfoxide, thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea.

Preferred non-heterocyclic ring cores include, but are not limited to, substituted or unsubstituted 1,3-cyclohexanedione, 1,3-cyclopentanedione; 1,3-dihydroxynaphthalene; or orthophenol.

Preferred heterocyclic cores include, but are not limited to, substituted or unsubstituted 3,7-dimethylxanthine, glutarimide, 3-methyl-7-pivoloylxanthine, methylthymine, methyluracil, 3-methylxanthine, tetrahydrophthalimide, thymine, uracil and xanthine, most preferably methyl-substituted xanthine. Exemplary preferred cores include, but are not limited to: C.sub.(1-6) alkyl-substituted thymine; C.sub.(1-6) alkyl-substituted uracil; 1,3-dihydroxynapthalene; 3,3-dimethylglutarimide; dihydrothymine; 2,4-dioxohexahydro-1,3,5-tetrazine; hexahydrophthalimide; homophthalimide; 2-hydroxypyridine; .beta.-ionone as vitamin A methylbarbituric acid; 2,6,6-methyl-1-cyclohexene-1-acetaldehyde as vitamin A; methyldihydroxypyrazolopyrimidine, specifically, 1,3-dimethyldihydroxypyrazolo[4,3-d]pyrimidine;1-methyl-5,6-dihydrouracil; 1,7-dimethylxanthine, 3,7-dimethylxanthine; 7-methylhypoxanthine; 1-methyllumazine; 3-methyl-7-methylpivaloylxanthine; methylpyrrolopyrimidine; 1-methylpyrrolo [2,3-d] pyrimidine; 1-methyl-2,4 (1H,3H)-quinolizinedione (1-methylbenzoyleneurea); methylthymine; 1-methyluracil; 3-methylxanthine; orotic acid; prostacyclin; 1-pyrrole amides; 2-pyrrole amides; 3-pyrrole amides; quinazolin-4(3H)-one; 1,2,3,4-tetrahydroisoquinoline; tetrahydrophthalimide; sulindac; uracil fused to naphthalene; 5- and/or 6-position substituted uracils (such as, for example, 5-bromouracil); tetralone to vitamin K; and 8-substituted xanthines (having substituents such as N or S).

Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimide, methylthymine, thymine, uracil or xanthine core. In representative, preferred compounds, R having formula I may be bonded to an N.sub.1 nitrogen of glutarimide; N.sub.1 nitrogen of xanthine (and N.sub.3 and N.sub.7 xanthine nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, C.sub.(1-6) alkyl, fluoro, chloro and amino); N.sub.3 nitrogen of methylthymine; or N.sub.1 nitrogen of uracil. Alternatively, R having formula I may be bonded to N.sub.1 and N.sub.3 xanthine nitrogens and N.sub.7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino. Representative, preferred inventive compounds are compounds of formulas II, III and IV: ##STR5## wherein R is defined above.

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier or diluent and some amount of an inventive compound. The compound may be present in an amount to effect a physiological response, or it may be present in a lesser amount such that the user will need to take two or more units of the composition to effect the treatment intended. These compositions may be made up as a solid, liquid or in a gaseous form. Or one of these three forms may be transformed to another at the time of being administered such as when a solid is delivered by aerosol means, or when a liquid is delivered as a spray or aerosol.

The nature of the composition and the pharmaceutical carrier or diluent will, of course, depend upon the intended route of administration, for example, parenterally, topically, orally or by inhalation for treatment of a patient with disease symptoms. For topical administration, the pharmaceutical composition will be in the form of a cream, ointment, liniment, lotion, paste, aerosol or drop suitable for administration to the skin, eye, ear, lung or nose. For parenteral administration, the pharmaceutical composition will be in the form of a sterile injectable liquid. For oral administration, the pharmaceutical composition will be in the form of a tablet, capsule, powder, pellet, atroche, lozenge, syrup, liquid, emulsion or aqueous or non-aqueous liquid suspension.

The invention includes a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune response or a cellular response to external or in situ primary stimuli. Treatment of the disease states involves mediating the cellular response through a specific phospholipid-based second messenger pathway acting adjacent to a cell membrane inner leaflet. The second messenger pathway is activated in response to various noxious or proliferative stimuli, characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. The inventive compounds are active by inhibiting various enzymes of this phospholipid second messenger pathway. The core moiety component of the inventive composition might serve to anchor the compound to an inner leaflet of a cell's plasma membrane allowing an "R" moiety of the inventive compound to interact with or inhibit an

enzyme involved in phospholipid metabolism, usually leading to cellular accumulation of specific PA (phosphatidic acid) species.

More specifically, the invention includes methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid and through glycan phosphatidylinostinol (Gly PI).

Illustrative examples of compounds of the present invention include, but are not limited to, the following:
##STR6## Method of Making the Inventive Compounds

The invention also provides a process for preparing the inventive compounds. The inventive process utilizes starting materials available to skilled artisans, whether commercially supplied or prepared from other materials commercially available. In addition, some, selected starting materials and intermediates available for use in the inventive process and a corresponding method of synthesis for these selected starting materials are disclosed in U.S. patent applications, Ser. Nos. 08/152,650 now U.S. Pat. No. 5,837,703 issued Nov. 17, 1998, and 08/164,081 now U.S. Pat. No. 5,470,878 issued Nov. 28, 1995, filed Nov. 12, 1993 and Dec. 8, 1993, respectively, the disclosures of which are incorporated in their entirety herein by reference.

The inventive carboxylic acid-, ester- and amide-substituted compounds of the invention may be prepared by the following general process. Specific, non-limiting examples of synthetic protocols for preparing exemplary compounds of the invention are set forth in the examples which follow.

In a method according to the invention, a compound containing a desired core (intended as a "core moiety" in the inventive compound) undergoes a reaction to produce an anion. Then, the resulting anion may be subsequently reacted with a suitable, substituted ester having at least one other functional group to displace a targeted functional group on the ester, thereby obtaining a compound according to the invention.

In a preliminary reaction, a predetermined amount of a core-containing compound is reacted with a base, a solvent and the suitable substituted ester to obtain an ester product. Again, the substituted ester has at least one functional group which may be substituted in a displacement reaction by the desired core-containing compound.

Preferred bases include, but are not limited to, sodium hydride, sodium amide, sodium alkoxide, lithium hydride, potassium hydride, lithium amide, sodium amide and potassium amide. An especially preferred base is sodium hydride. Preferred solvents may be dimethylsulfoxide, dimethylformamide, or an alcohol. An alcohol may be chosen from among methanol, ethanol or isopropanol. Any substituted ester comprising a chain structure of the inventive compounds may be used in this preliminary reaction, as long as a functional group is present for displacement. Preferred esters may be substituted esters and may be, but are not limited to, halo-substituted esters.

These ester products, which have a composite structure of a core-moiety and ester-containing side chain may then subsequently be converted to an inventive compound having a carboxylic acid-substituted side chain.

In this process, the ester product is reacted with an ester-hydrolyzing agent to obtain an inventive compound having a carboxylic acid-substituted side chain. Representative ester-hydrolyzing agents useful in preparing inventive carboxylic acid-containing inventive compounds may be potassium hydroxide or sodium hydroxide in water, although other ester-hydrolyzing agents are within the scope of the inventive process.

In a halogenation reaction, the carboxylic acid-containing compound above may be reacted with a halogenating agent to obtain an intermediate having a carboxylic acid halide functional group. Although other agents are within the scope of the inventive method, halogenating agents may be chosen from among thionyl chloride, phosphorus trichloride, phosphorus pentachloride, phosphorus oxychloride, thionyl bromide and the like.

Once the intermediate prepared in the step above, containing a carboxylic acid halide functional group is isolated, it is then be reacted with an amine to obtain a corresponding amide-containing inventive compound. In this reaction, the amine compound will contribute to a portion of the final structural configuration of the inventive amide-containing compounds.

Alternatively, a compound containing a desired core may be reacted with a base and substituted-olefin, producing an intermediate olefinic product. The substituted olefin starting material will have a target

functional group which will be displaced by an anion of the core-containing compound. In this reaction, a predetermined amount of a core-containing compound is reacted with a suitable base, a solvent and a substituted olefin. Again, the substituted olefin has at least one functional group for displacement.

Preferred bases include, but are not limited to, sodium hydride, sodium amide, sodium alkoxide, lithium hydride, potassium hydride, lithium amide, sodium amide, potassium amide and sodium hydride. Preferred solvents may be dimethylsulfoxide, dimethylformamide, or an alcohol such as, for example, methanol, ethanol or isopropanol. Any substituted olefin comprising a chain structure of the inventive compounds may be used in the preliminary reaction according to the invention. Preferred olefins may be substituted olefins. Preferred substituted olefins include, but are not limited to halo-substituted olefins.

By reacting the intermediate olefinic product previously obtained with an oxidizing agent, a diol is prepared from the olefinic product. Preferred oxidizing agents include, but are not limited to, osmium tetroxide. Preferred oxidizing agents, such as osmium tetroxide may require a catalytic amount of the oxidizing agent in the presence of a regenerating agent. Representative regenerating agents may be 4-methylmorpholine-N-oxide and trimethylamine-N-oxide. An especially preferred regenerating agent is 4-methylmorpholine-N-oxide. In a subsequent halogenation reaction, the resulting diol is converted to an inventive compound using a halogenating agent in the presence of an organic acid. Exemplary halogenating agents include, but are not limited to, hydrogen bromide and hydrogen chloride. Preferred organic acids may be acetic acid and propionic acid.

Also, inventive amide- and ester-substituted compounds according to the invention may also be prepared by reacting a compound containing at least one of an alcohol or amine functional group with a substituted acyl halide or carboxylic acid anhydride. The compound containing at least one alcohol or amine also has as a structural component a core moiety corresponding to a core moiety of the inventive compounds. Starting materials may be obtained commercially or by synthesis from other materials which are commercially available. Some amino alcohol compounds may also be prepared as disclosed in the above-identified copending U.S. patent applications.

A schematic representation of an inventive process for preparing an amide-substituted inventive compound is illustrated as follows: ##STR7##

inventive amide-containing compound

Uses of the Invention Compounds and Pharmaceutical Formulations

The inventive compounds provide a method for maintaining homeostasis in cells contacted by primary stimuli by mitigating the effects of these primary stimuli on the secondary signaling pathways invoked within seconds of a primary stimulus. For example, administration of an inventive compound in vivo or ex vivo provides a method to modify cellular behavior, the method comprising contacting cells (in vivo or ex vivo), whose behavior is to be modified, with an effective amount of an inventive compound or a pharmaceutical composition thereof. The method is a method to: (1) inhibit proliferation of tumor cells, being; (2) suppress activation of T-cells by antigen or IL-2 stimulation being; (3) suppress activation of monocyte/macrophage cells by endotoxin, TNF, IL-1 or GM-CSF stimulation, being; (4) suppress antibody production of B-cells in response to an antigen, IL-4 or CD40 ligand, being; (5) inhibit the proliferation of smooth muscle cells in response to growth factors capable of stimulating said proliferation, being; (6) lower systemic vascular resistance conferred by endothelial cells, being; (7) lower systemic vascular resistance induced by endothelial cells, being; (8) lower expression of adhesion molecules induced by enhancers thereof, being; (9) suppress the activation of T-cells and macrophages by HIV, being; (10) inhibit the proliferation of kidney mesangial cells in response to stimulation by IL-1 and/or MIP-1.alpha. and/or PDGF and/or FGF, being; (11) enhance the resistance of kidney glomerular or tubular cells to cyclosporin A or amphotericin B, being; (12) prevent the release of MIP-1 by IL-1, TNF, or endotoxin stimulated monocytes and macrophages; (13) prevent the release of platelet activating factor by IL-1, TNF, or endotoxin treated megakaryocytes, fibroblastic cells, and macrophages; (14) prevent the down-regulation of receptors for cytokines in TNF-treated hematopoietic progenitor cells, being; (15) suppress the production of metalloproteases in IL-1-stimulated or TNF-stimulated glomerular epithelial cells or synovial cells, being; (16) enhance the resistance of gastrointestinal or pulmonary epithelial cells to cytotoxic drugs or radiation, being; (17) enhance the antitumor effect of a non-alkylating antitumor agent, being; (18) to inhibit the production of osteoclast activating factor in response to IL-1, being; (19) inhibit degranulation in response to IgE, being; (20) enhance the release of adrenergic neural transmitters, dopamine, norepinephrine, or epinephrine, or the neurotransmitter, acetylcholine, being; (21) modulate the post-synaptic "slow current" effects of the adrenergic neurotransmitters dopamine, epinephrine, or norepinephrine, or the neurotransmitter acetylcholine, being; (22) suppress signaling by

neurotransmitters including acetyl choline, leuenkephalin and serotonin; or (23) increase seizure threshold.

Indications useful for administering compounds of the invention include, but are not limited to: the presence of a tumor burden, a hormone-related disorder, a neurological disorder, an autoimmune disease, inflammation, restenosis, coronary artery disease, atherosclerosis, hypertension, unwanted immune response (such as allograft reactions), viral infection, nephritis, mucositis, and various allergic responses. Allergic responses include, but are not limited to, acute allergic response and thus rhinorrhea, sinus drainage, diffuse tissue edema, and generalized pruritus. As well as the following, other chronic allergic responses include, but are not limited to, dizziness, diarrhea, tissue hyperemia, and lacrimal swelling with localized lymphocyte infiltration. Allergic reactions are also associated with leukotriene release and the distal effects thereof, including asthmatic symptoms (e.g., development of airway obstruction, a decrease in FEV1, changes in vital capacity, and extensive mucus production).

Other suitable subjects for the administration of compounds of the invention, include patients: being administered other cytotoxic agents for the treatment of tumors, such as chemotherapeutic agents or irradiation therapy; suffering from neoplasias generally, whether or not otherwise treated including acute and chronic myelogenous leukemia, hairy cell leukemia, lymphomas, megakaryocytic leukemia, and the like; disease states caused by bacterial, fungal, protozoal, or viral infection; exhibiting unwanted smooth muscle cell proliferation in the form of, for example, restenosis, such as patients undergoing cardiac surgery; afflicted with autoimmune diseases, thus requiring deactivation of T and B cells, and having neurological disorders.

The compounds of the invention further are able to decrease enhanced levels of a relevant PA and DAG resulting from stimulation of synaptosomes with acetylcholine and/or epinephrine. This suggests that the effects of the compounds of the invention are to both enhance the release of inhibitory neural transmitters such as dopamine, and to modulate the distal "slow current" effects of such neurotransmitters.

Thus, the drugs of the invention are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent the toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and generally improve memory in subjects with organic deficits, including Alzheimer's patients.

While dosage values will vary, therapeutic efficacy is achieved when the compounds of the invention are administered to a human subject requiring such treatment as an effective oral, parenteral, or intravenous sublethal dose of about 50 mg to about 5000 mg per day, depending upon the weight of the patient. A particularly preferred regimen for use in treating leukemia is 4-50 mg/kg body weight. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual's need and to the professional judgment of the person administering or supervising the administration of the inventive compounds.

Pharmaceutical Formulations

A suitable formulation will depend on the nature of the disorder to be treated, the nature of the medicament chosen, and the judgment of the attending physician. In general, the inventive compounds are formulated either for injection or oral administration, although other modes of administration such as transmucosal or transdermal routes may be employed. Suitable formulations for these compounds can be found, for example, in Remington's Pharmaceutical Sciences (latest edition), Mack Publishing Company, Easton, Pa.

The inventive compounds and their pharmaceutically acceptable salts can be employed in a wide variety of pharmaceutical forms. The preparation of a pharmaceutically acceptable salt will be determined by the chemical nature of the compound itself, and can be prepared by conventional techniques readily available. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1 gram, wherein the amount of inventive compound per dose will vary from about 25 mg to about 1 gram for an adult. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension. Where the inventive composition is in the form of a capsule, any routine encapsulation is suitable,

for example, using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule, any pharmaceutical carrier routinely used for preparing dispersions of suspensions may be considered, for example, aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell. A syrup formulation will generally consist of a suspension or solution of the compound or salt thereof in a liquid carrier (e.g., ethanol, polyethylene glycol, coconut oil, glycerine or water) with a flavor or coloring agent.

The amount of inventive compound required for therapeutic effect on topical administration will, of course, vary with the compound chosen, the nature and severity of the disease and the discretion of the treatment provider. Parenteral includes, but is not limited to, intravenous, intramuscular, subcutaneous, intranasal, intrarectal, intravaginal or intraperitoneal administration. Appropriate dosage forms for such administration may be prepared by conventional techniques. A typical parenteral composition consists of a solution or suspension of the inventive compound or a salt thereof in a sterile or non-aqueous carrier, optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil. The daily dosage for treatment of sepsis or another severe inflammatory condition via parenteral administration is suitable from about 0.001 mg/kg to about 40 mg/kg, preferably from about 0.01 mg/kg to about 20 mg/kg of an inventive compound or a pharmaceutically acceptable salt thereof calculated as the free base.

The inventive compounds may be administered orally. The daily dosage regimen for oral administration is suitably from about 0.1 mg/kg to about 1000 mg/kg per day. For administration the dosage is suitably from about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit activity.

The inventive compounds may be administered by inhalation (e.g., intranasal or oral). Appropriate dosage forms include, but are not limited to, an aerosol or a metered dose inhaler, as prepared by conventional techniques. The daily dosage is suitably from about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base. Typical compounds for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant.

The invention is illustrated by the following examples which should not be regarded as limiting the invention in any way.

EXAMPLE 1

This example is a synthesis for inventive compound no. 1527 (see above for chemical name and structure). A mixture of theobromine (1.0 g, 5.5 mmol, available from Sigma) and a solution (20 ml) of 50% sodium hydride in oil (264 mg, 5.5 mmol) in dimethylsulfoxide was stirred for 50 minutes, followed by addition of 6-bromo-1-hexanol (1.0 g, 5.5 mmol, available from Aldrich). After stirring for 18 hours, the solution was treated with 50 ml of water and then extracted with two 25 ml aliquots of hexanes. The aqueous phase was extracted with three 35 ml aliquots of 25% ethanol-dichloromethane. The combined ethanol-dichloromethane extracts were dried over magnesium sulfate and then the solvents were evaporated under vacuum. The remaining dimethylsulfoxide was removed by distillation under full pump vacuum, producing 1.4 g of a white powder, 1-(6-hydroxyhexyl)-3,7-dimethylxanthine (5.0 mmol, 91% yield).

A solution (5 ml) of chloroacetyl chloride (339 mg; 3 mmol) in dichloromethane was added dropwise at 0.degree. C. to a solution (5 ml) of 1-(6-hydroxyhexyl)-3,7-dimethylxanthine (560 mg; 2 mmol) and triethylamine (607.2 mg; 6 mmol) in dichloromethane. The reaction was slowly warmed to room temperature and stirred overnight. The reaction was quenched with saturated sodium bicarbonate solution (5 ml) and extracted with three 50 ml aliquots of dichloromethane. The combined organic extracts were washed with 1% dilute hydrogen chloride (15 ml), followed by water (15 ml) and finally with brine solution (15 ml), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. A crude product obtained was further purified by flash chromatography over silica gel using a 20% hexane/ethyl acetate eluant, resulting in 296 mg of compound no. 1527 (50.1% yield).

EXAMPLE 2

Theobromine (11.9 g, 66 mmol, available from Sigma) was added to a mixture of bromohexene (10.7 g, 66 mmol, available from Aldrich) and sodium hydride (1.58 g, 66 mmol) in dimethylsulfoxide (100 ml) and the resulting mixture stirred for 43 hours. The solution was treated with water (200 ml) and then extracted with

three 80 ml aliquots of dichloromethane. The combined extracts were washed with three 100 ml aliquots of water and dried over magnesium sulfate. The solvent was evaporated under vacuum, leaving 17 g of a white powder, 1-(5-hexenyl)-3,7-dimethylxanthine (65 mmol, 98% yield).

Six drops of 2.5% osmium tetraoxide in t-butanol were added to a mixture of 1-(5-hexenyl)-3,7-dimethylxanthine (1.07 g, 4.1 mmol), as prepared above and N-methylmorpholine-N-oxide (1.44 g, 12.3 mmol) in water (20 ml) and acetone (10 ml). After stirring the resulting mixture for 48 hours, the mixture was treated with 20% aqueous sodium dithionite solution (20 ml). After 2 minutes, the mixture was extracted with three 30 ml aliquots of a 25% ethanol-dichloromethane solution. The combined extracts were dried over magnesium sulfate and the solvent was evaporated under vacuum, leaving 750 mg of a white powder, 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine (2.53 mmol, 62% yield).

A solution of 1-(5,6-dihydroxyhexyl)-3,7-dimethylxanthine (0.50 g, 1.7 mmol) and 1,1'-carbonyldiimidazole (1.10 g, 6.8 mmol) was refluxed for 20 hours. Water (30 ml) was added and the mixture was extracted with three 50 ml aliquots of dichloromethane. The combined organic layers were washed with two 30 ml aliquots of water and dried over sodium sulfate. The solvent was removed under vacuum. A residue was further purified by chromatography over silica using an ethyl acetate-10% ethanol eluant, yielding 180 mg of compound no. 1578 (33% yield).

EXAMPLE 3

Methanesulfonyl chloride (2.20 g, 1.5 ml, 19.2 mmol) was added to a solution (100 ml) of 9-decene-1-ol (3.00 g, 19.2 mmol, available from Aldrich) in dichloromethane at 0.degree. C., followed by addition of triethylamine (2.91 g, 28.8 mmol). After stirring was continued for 15 minutes at 0.degree. C., the reaction was allowed to warm to room temperature. After 2 hours, the reaction mixture was poured into water (100 ml) and extracted with three 60 ml aliquots of dichloromethane. The combined organic portions were dried over sodium sulfate and the solvent was evaporated under vacuum, leaving a yellow oil mesylate (4.52 g, 100%), which was used without further purification.

Theobromine (3.45 g, 19.2 mmol) was added to a suspension (30 ml) of sodium hydride (461 mg, 19.2 mmol) in dimethylsulfoxide. After 15 minutes, the 9-decenylmesylate (2.25 g, 11 mmol) was added and the reaction stirred for 18 hours at 25.degree. C., then for 40 minutes at 100.degree. C. The mixture was then poured into water (100 ml) and extracted with three 50 ml aliquots of dichloromethane. The combined organic portions were washed with saturated salt solution (60 ml) and dried over magnesium sulfate. Evaporating the solvent under vacuum left a white solid residue. Recrystallization of the residue in ether produced 3.40 g of 1-(9-decenyl)-3,7-dimethylxanthine (56% yield). 1-(9-Decenyl)-3,7-dimethylxanthine (3.2 g, 10.1 mmol), 4-methylmorpholine-N-oxide (1.41 g, 12 mmol), 3 drops of 2.5% osmium tetroxide solution in t-butanol, acetone (40 ml) and water (10 ml) were stirred for 24 hours. Following addition of 5 ml of a saturated solution of sodium dithionite and a further 15 minutes of stirring, the reaction mixture was extracted with four 50 ml aliquots of 25% ethanol/dichloromethane. The combined organic portions were dried over sodium sulfate. Evaporating the solvents left a white solid residue, which upon recrystallization in ethanol produced 3.3 g of 1-(9,10-dihydroxydecyl)-3,7-dimethylxanthine (93% yield). 1-(9,10-Dihydroxydecyl)-3,7-dimethylxanthine (2.11 g, 6 mmol), prepared above, was stirred with hydrogen bromide (3.58 ml, 4.85 g of a 30% solution in acetic acid, 18 mmol) for 90 minutes. The mixture was then added to a flask containing 40 ml aqueous sodium bicarbonate solution (5 g) and 50 ml dichloromethane. After 10 minutes of vigorous stirring the layers were separated and the aqueous portion washed with two 50 ml aliquots of dichloromethane. The organic portions were combined, dried over sodium sulfate, and evaporating the solvent produced 2.72 g of a yellow oil, inventive compound no. 1583 (100% yield).

EXAMPLE 4

Sodium hydride (343 mg, 14 mmol) was added to a stirring solution of 1-methylthymine (2.00 g, 14 mmol) in dimethylsulfoxide (40 ml). After 15 minutes, 9-bromo-1-nonene (2.93 g, 14 mmol, available from Alfebro) was added and the resulting mixture stirred for 20 hours. The reaction was poured into water (40 ml) and extracted with three 50 ml aliquots of dichloromethane. The organic layers were combined, washed with water (40 ml) and saturated aqueous salt solution (20 ml). After drying the washed organic layers over sodium sulfate, the solvent was evaporated, leaving a colorless oil, 3-(8-nonenyl)-1-methylthymine, which solidified upon standing (2.76 g, 73% yield).

A solution of 3-(8-nonenyl)-1-methylthymine (2.63 g, 9.9 mmol), prepared above, 4-methylmorpholine-N oxide (1.39 g, 12 mmol), and potassium osmate (IV) dihydrate (7 mg, 2.times.10.sup.-5 mol) in acetone (20 ml) and water (10 ml) was stirred for 18 hours. After addition of a saturated aqueous solution of sodium

hydrosulfite (10 ml) and 15 minutes of stirring, the reaction mixture was extracted with dichloromethane (50 ml) and with two 50 ml aliquots of dichloromethane/20% methanol. The combined organic layers were washed with water (15 ml) and saturated aqueous salt solution (15 ml), and then dried over sodium sulfate. The solvent was evaporated under vacuum, leaving a white solid residue. Recrystallization of the solid in ethanol yielded 2.68 g of 3-(8,9-dihydroxynonyl)-1-methylthymine (91% yield).

A mixture of 3-(8,9-dihydroxynonyl)-1-methylthymine (2.16 g, 7.6 mmol), prepared above, and a 30% solution of hydrogen bromide in acetic acid (4.5 ml, 23 mmol) was stirred for 1 hour. The reaction mixture was added slowly to a beaker containing sodium bicarbonate (8.4 g, 0.1 mol), ice water (30 ml), and dichloromethane (30 ml). The layers were separated, and the aqueous layer extracted with two 60 ml aliquots of dichloromethane. The combined organic layers were washed with water (30 ml) and saturated aqueous salt solution (30 ml). The washed organic layers were then dried over sodium sulfate. Evaporation of the solvent produced 2.59 g of a slightly orange oil, inventive compound no. 1908 (85% yield).

EXAMPLE 5

This example is a method of synthesis for inventive compound no. 2573 (see above for chemical name and compound). A mixture of theobromine (17.64 g, 98 mmol) and sodium hydride (2.35 g, 98 mmol) in dimethylsulfoxide (250 ml) was stirred for 15 minutes. After addition of 9-bromo-1-nonene (20.0 g, 98 mmol, available from Alfebro) stirring was continued at ambient temperature for 3 days. The reaction mixture was then poured into water (300 ml) and extracted with four 200 ml aliquots of dichloromethane. The combined organic layers were washed with two 150 ml aliquots of saturated aqueous salt solution and the washed layers dried over sodium sulfate. Evaporating the solvent under vacuum resulted in a thick oil, which resulted in 24.34 g of white crystals after cooling a solution of the thick oil in a minimum of dichloromethane and ether 1-(8-nonenyl)-3,7-dimethylxanthine (77.5 mmol, 99% yield).

A solution of 1-(8-nonenyl)-3,7-dimethylxanthine (810 mg, 2.7 mmol), prepared above, 4-methylmorpholine-N-oxide (340 mg, 2.9 mmol) and 3 drops of 2.5% osmium tetroxide in t-butanol, acetone (20 ml) and water (20 ml) was stirred for 24 hours, followed by addition of saturated aqueous sodium dithionite solution (5 ml). After stirring the resulting mixture for 15 minutes, the reaction mixture was extracted with four 50 ml aliquots of 25% ethanol-dichloromethane. The combined organic layers were dried over sodium sulfate, and the solvent evaporated under vacuum. A resulting solid residue was recrystallized in ethanol-chloroform, producing 490 mg of 1-(8,9-dihydroxynonyl)-3,7-dimethylxanthine (54% yield).

A mixture of 1-(8,9-dihydroxynonyl)-3,7-dimethylxanthine, prepared above, and 30% hydrogen bromide in acetic acid (0.8 ml, 3.90 mmol) was stirred for 90 minutes. The solution was poured into a mixture of water (10 ml), sodium bicarbonate (1.35 g, and dichloromethane (10 ml). After 10 minutes of vigorous stirring, the layers were separated and the aqueous portion was extracted with three 15 ml aliquots of dichloromethane. The combined organic phases were dried over sodium sulfate and the solvent was evaporated under vacuum, leaving 550 mg of a yellow oil, 1-(8-acetoxy-9-bromononyl)-3,7-dimethylxanthine (96% yield). Without further purification, the oil was dissolved in methanol (5 ml), to which a 1 M solution of sodium methoxide in methanol (4.1 ml, 4.1 mmol) was added. After 30 minutes, the reaction mixture was poured into water (30 ml) was extracted with three 40 ml aliquots of dichloromethane. The combined organic layers were dried over sodium sulfate. Evaporating the solvents under vacuum left a solid residue. Recrystallization in dichloromethane-petroleum ether yielded 380 mg of 1-(8,9-oxidononyl)-3,7-dimethylxanthine (91% yield).

A mixture of 1-(8,9-oxidononyl)-3,7-dimethylxanthine (0.50 g, 1.6 mmol), prepared above and lithium perchlorate (166 mg, 1.6 mmol) was stirred in anhydrous acetonitrile (40 ml). After addition of dodecylamine (1.48 g, 8.0 mmol, available from Aldrich), the mixture was stirred at reflux for 4 hours. After cooling, dichloromethane (50 ml) was added and the mixture was washed with water (30 ml) and saturated aqueous salt solution (30 ml), and then dried over sodium sulfate. The solvent was removed under vacuum, leaving a white residue. Further purification by chromatography over silica using a dichloromethane/5%methanol eluant, produced 263 mg of a white solid, inventive compound no. 2573 (33% yield).

EXAMPLE 6

This example is a method of synthesis for inventive compound no. 3508. Triphenylphosphine (5.24 g, 20 mmol) was added incrementally to a solution of oleyl alcohol (5.37 g, 20 mmol) and carbontetrabromide (6.63 g, 20 mmol) in 400 ml of dichloromethane, the resulting reaction mixture being stirred for an hour at room temperature. Removing the solvent under reduced pressure, left a residue, which was extracted with three 200 ml aliquots of hexane. Further purification by flash chromatography over silica gel using a hexane eluant produced 5.82 g of 1-bromo-9-octadecene (88% yield).

Sodium hydride (95%, 84 mg, 3.5 mmol) was added to a solution of theobromine (0.595 g, 3.2 mmol) in dimethylsulfoxide (15 ml). After 20 minutes of stirring, 1-bromo-9-octadecene (0.995 g, 3 mmol), prepared above, was added. After 6 hours of stirring at room temperature, the reaction mixture was warmed to 60.degree. C. over 3 hours and then poured into a separatory funnel containing 50 ml of water. The reaction mixture was extracted with five 40 ml aliquots of dichloromethane. The organic extracts were combined, washed with water (50 ml) and brine (50 ml) and dried over anhydrous magnesium sulfate. Removing the solvent under reduced pressure resulted in a crude product further purified by flash chromatography over silica gel using a 30% acetone/petroleum ether eluant, yielding 0.44 g of 1-(9-octadecenyl)-3,7-dimethylxanthine (34% yield).

A solution of 1-(9-octadecenyl)-3,7-dimethylxanthine (0.15 g, 0.35 mmol), 4-methylmorpholine-N-oxide (49 mg, 0.42 mmol, 1.2 equivalents.) and potassium osmate dihydrate (1 mg) in acetone (4 ml) and water (1 ml) was stirred for 6 hours. A solution of 20% aqueous sodium sulphite (2 ml) was added and stirred for 30 minutes. The reaction mixture was extracted with four 10 ml aliquots of 25% ethanol/dichloromethane. The combined organic extracts were dried over anhydrous magnesium sulfate, the solvent evaporated under reduced pressure and a residue purified by flash chromatography over silica gel using a methanol (5%)/dichloromethane eluant, yielding 0.65 g of 1-(9,10-dihydroxyoctadecyl)-3,7-dimethylxanthine (40.4% yield).

A 50 ml RB flask fitted with a dropping funnel, magnetic stirring bar and an argon inlet was placed in a solution of 1-(9,10-dihydroxyoctadecyl)-3,7-dimethylxanthine (464 mg; 1 mmol) and triphosgene (148.37 mg; 0.5 mmol) in anhydrous dichloromethane. The resulting mixture was cooled to 0.degree. C. A solution of pyridine (58.2 mg; 2 mmol) in anhydrous dichloromethane (3 ml) was added dropwise and the reaction mixture was warmed to room temperature and stirred for 6 hours. The reaction mixture was then diluted with water (20 ml) and extracted with three 50 ml aliquots of dichloromethane. The combined organic extract was washed with water (50 ml), saturated copper sulphate solution (50 ml), water (50 ml), and brine solution (50 ml) and dried over anhydrous magnesium sulfate. Evaporating the solvent under reduced pressure left a residue which was further purified by flash chromatography over silica gel using a 50% ethyl acetate/hexane eluant, resulting in 200 mg of compound no. 3508 (40.8% yield).

EXAMPLE 7

This example is a method of synthesis for inventive compound no. 3537. Sodium hydride (95%, 1.26 g, 50 mmol) was added to a solution of theobromine (7.2 g, 40 mmol) in dimethylsulfoxide (300 ml). After 20 minutes of stirring, undecenylmesylate (7.95 g, 30 mmol) was added and the resulting mixture stirred for 12 hours at room temperature. The reaction was warmed to 70-80.degree. C. and stirred for 4 hours. The reaction mixture was then poured into a separatory funnel containing water (1 L) and extracted with five 200 ml aliquots of dichloromethane. The organic extracts were combined, washed with water (100 ml) and brine (100 ml) and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, resulting in a crude product, which was further purified by flash chromatography over silica gel using a 20% hexane/dichloromethane eluant producing 4.6 g of 1-(10-undecenyl)-3,7-dimethylxanthine (46.3% yield).

A solution of 1-(10-undecenyl)-3,7-dimethylxanthine (4.3 g, 13 mmol), prepared above, 4-methylmorpholine-N-oxide (1.942 g, 16.6 mmol) and potassium osmate dihydrate (9.5 mg, 0.026 mmol) in acetone (45 ml) and water (10 ml) was stirred for 6 hours. A solution of 20% aqueous sodium sulphite (12 ml) was added and stirred for 30 minutes. The reaction mixture was extracted with four 100 ml aliquots of 25% ethanol/dichloromethane. The combined organic extracts were dried over anhydrous magnesium sulfate. Evaporating the solvent under reduced pressure left a residue, which upon subsequent purification by flash chromatography over silica gel using a methanol (5%)/dichloromethane eluant produced 3.6 g of 1-(10,11-dihydroxyundecanyl)-3,7-dimethylxanthine (76% yield).

1-(10,11-Dihydroxyundecanyl)-3,7-dimethylxanthine (3.6 g, 10 mmol) was stirred with hydrogen bromide (6.2 ml, 8.4 g of a 30% solution in acetic acid, 31.1 mmol) for 90 minutes. The mixture was then added to a flask containing 100 ml aqueous sodium bicarbonate solution and 75 ml dichloromethane. After 10 minutes of vigorous stirring, the layers were separated and the aqueous portion washed with three 75 ml aliquots of dichloromethane. The organic portions were combined and dried over magnesium sulfate. Evaporating the solvent left 3.6 g of 1-(10-acetoxy-11-bromoundecanyl)-3,7-dimethylxanthine. Without further purification, 1-(10-acetoxy-11-bromoundecanyl)-3,7-dimethylxanthine was taken up in 25 ml of methanol and treated with a solution of sodium methoxide (prepared from 0.28 g, 12.2 mmol sodium, and 25 ml methanol). After 30 minutes, most of the solvent was removed under reduced pressure and the residue was extracted with three 75 ml aliquots of dichloromethane. The organic portions were combined and dried over magnesium sulfate.

The solvent was evaporated under reduced pressure, leaving an off-white solid. Further purification of the off-white solid by column chromatography over silica gel using a dichloromethane/(3%) methanol eluant provided 2.0 g of 1-(10,11-oxidoundecanyl)-3,7-dimethylxanthine (57.5% yield).

Octylamine (3.4 ml, 21 mmol) was added to a stirring mixture of 1-(10,11-oxidoundecyl)-3,7-dimethylxanthine (5.00 g, 14.4 mmol), prepared above, and lithium perchlorate (1.69 g, 16 mmol) in anhydrous acetonitrile (60 ml). Stirring was continued for 16 hours at 50.degree. C. After cooling to ambient temperature, water (100 ml) was added, and the mixture was extracted with three 100 ml aliquots of dichloromethane-10% methanol. The combined organic extracts were washed with aqueous saturated salt solution (150 ml) and dried over sodium sulfate. The solvent was removed under vacuum, leaving a solid, which was recrystallized twice in dichloromethane/ether/hexane, producing 5.76 g of a white powder, 1-(11-octylamino-10-hydroxyundecyl)-3,7-dimethylxanthine (84% yield).

A solution of 1-(11-octylamino-10-hydroxyundecyl)-3,7-dimethylxanthine (1.70 g, 3.0 mmol), prepared above, in acetic anhydride (5 ml) was heated for 2 hours at 90.degree. C. After cooling, methanol (10 ml) was added and the mixture was stirred for 30 minutes. After addition of water (20 ml), the mixture was extracted with three 40 ml aliquots of dichloromethane. The combined organic layers were washed with water (15 ml) and saturated aqueous salt solution (15 ml). After the solution was dried over sodium sulfate, the solvent was evaporated, leaving a yellow oil residue. The residue was further purified by chromatography over neutral activity II alumina using a dichloromethane-5% methanol eluant, resulting in 1.43 g of a colorless oil, inventive compound no. 3537 (2% yield), which solidified upon standing.

EXAMPLE 8

This example is a method of synthesis for inventive compound no. 3541 (see above for chemical name and structure). Sodium hydride (312 mg, 13 mmol) was added to a solution of octanol (10 ml) in toluene (20 ml). After bubbling ceased, 1-(10,11-oxidoundecanyl)-3,7-dimethylxanthine (2.50 g, 7.2 mmol), prepared in example 7 above, was added to the mixture, which was subsequently stirred for 3 hours at 60-70.degree. C. After cooling, the mixture was added to a solution of saturated aqueous solution of ammonium chloride (15 ml) and water (10 ml) and extracted with three 50 ml aliquots of dichloromethane. The combined organic layers were washed with saturated aqueous salt solution and dried over sodium sulfate. Evaporation of the solvents under vacuum left a solid residue, which when purified by chromatography over neutral activity II alumina using a dichloromethane eluant produced recovered epoxide (411 mg) and 1.34 g of 1-(11-octyloxy-10-hydroxyundecyl)-3,7-dimethylxanthine (49% yield).

A mixture of 1-(11-octyloxy-10-hydroxyundecyl)-3,7-dimethylxanthine (0.31 g, 0.6 mmol), prepared above, and acetic anhydride (4 ml) was heated at 90.degree. C. for 2 hours. After cooling to ambient temperature, dichloromethane (40 ml) and saturated sodium bicarbonate solution (50 ml) were added. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (50 ml). The combined organic layers were washed with water (10 ml) and saturated aqueous salt solution (10 ml). After the solution was dried over sodium sulfate, the solvent was removed, leaving an oily residue. The residue was purified by chromatography over silica using a dichloromethane-10% methanol eluant, producing 149 mg of inventive compound no. 3541 (49% yield).

EXAMPLE 9

This example is a method of synthesis for inventive compound no. 3549 (see above for chemical name and structure). A solution of 11-bromoundecanoic acid (5.70 g, 22 mmol, available from Aldrich) and p-toluenesulfonic acid (0.1 g) in absolute ethanol (100 ml) was refluxed for 3 hours. A saturated aqueous sodium bicarbonate solution (40 ml) was added and the reaction mixture then extracted with three 70 ml aliquots of dichloromethane. The combined extracts were washed with water (50 ml) and saturated aqueous salt solution (50 ml) and the solvent was evaporated, leaving a colorless oil. Ethyl 11-bromoundecanoate (5.92 g, 94% yield) was collected during distillation (2 mm) at 135.degree. C. A solution of this bromoester (5.92 g, 20 mmol) and 1-sodiotheobromine (4.08 g, 20 mmol) in dimethylsulfoxide (80 ml) was stirred for 18 hours at ambient temperature. The mixture was added to water (100 ml) and dichloromethane (100 ml). The aqueous layer was extracted with two 80 ml aliquots of dichloromethane. The combined organic layers were washed with water (80 ml) and saturated aqueous salt solution (80 ml), dried over magnesium sulfate, and the solvent was evaporated under vacuum, leaving a white solid residue. The residue was recrystallized in dichloromethane/ether/hexane, yielding 4.95 g of 1-(ethyl 11-yl-undecanoate)-3,7-dimethylxanthine (62% yield).

A solution of potassium hydroxide (0.50 g, 9.0 mmol) in water (1 ml) was added to a stirring suspension of 1-

(ethyl 11-yl-undecanoate)-3,7-dimethylxanthine (2.52 g, 6.4 mmol), prepared above, in methanol (15 ml). The mixture was warmed until homogeneous, and the stirring was continued overnight at ambient temperature. Water (10 ml) was added to the reaction mixture, followed by a 5% solution of sulfuric acid (10 ml). The precipitate was filtered off and washed with ether, then dried under vacuum, resulting in 2.12 g of inventive compound no. 3549 (91% yield).

EXAMPLE 10

This example is a method of synthesis for inventive compound no. 3554 (see above for chemical name and structure). A solution of 1-(11-yl-undecanoic acid)-3,7-dimethylxanthine (1.62 g, 4.5 mmol), prepared in example 10 above, and thionyl chloride (0.5 ml, 6.7 mmol) in toluene (5 ml) was heated at 80.degree. C. for 1 hour and then cooled. The solvent was evaporated under a nitrogen stream. The resulting acid chloride was taken up in dichloromethane (20 ml), and 1-octylamine (2 ml, 11 mmol) was added by syringe to the stirring solution. After 2 hours, water (50 ml) was added and the mixture was extracted with three 50 ml aliquots of dichloromethane. The combined organic extracts were washed with 5% hydrochloric acid (100 ml) and saturated aqueous salt solution (60 ml) and then dried over sodium sulfate. The solvent was evaporated under vacuum, leaving a residue, which was further purified by chromatography over basic activity II alumina using a dichloromethane/10% methanol eluant, yielding 1.47 g of compound no. 3554 as a white solid (69% yield).

EXAMPLE 11

This example is a method of synthesis for inventive compound no. 3564 (see above for chemical name and structure). Tetradecylamine (797 mg, 3.7 mmol) was added to a stirring mixture of 1-(10,11-oxidoundecanyl)-3,7-dimethylxanthine (1.00 g, 2.9 mmol), prepared in example 7 above, and lithium perchlorate (309 mg, 2.9 mmol) in anhydrous acetonitrile (20 ml). Stirring was continued for 4 hours at 60.degree. C. After cooling to ambient temperature, water (50 ml) was added, and the mixture was extracted with three 100 ml aliquots of dichloromethane. The combined organic extracts were washed with aqueous saturated salt solution and dried over sodium sulfate. The solvent was removed under vacuum, leaving a solid residue, which was purified by chromatography over neutral activity II alumina using a dichloromethane-3% methanol eluant, resulting in 550 mg of a white powder, 1-(11-tetradecylamino-10-hydroxyundecyl)-3,7-dimethylxanthine (34% yield).

A solution of 1-(11-tetradecylamino-10-hydroxyundecyl)-3,7-dimethylxanthine (600 mg, 1.1 mmol) and acetic anhydride (0.6 ml, 6.4 mmol) in pyridine (15 ml) was stirred at ambient temperature for 20 hours. After addition of dichloromethane (100 ml) the mixture was washed with two 50 ml aliquots of 10% aqueous hydrochloric acid and saturated aqueous salt solution (50 ml), and then dried over magnesium sulfate. The solvent was removed under vacuum, leaving a residue, which was then purified by chromatography over neutral activity II alumina using a dichloromethane-3% methanol eluant, resulting in 475 mg of inventive compound no. 3564 (69% yield).

EXAMPLE 12

This example is a method of synthesis for inventive compound no. 3577 (see above for chemical name and structure). Under an argon atmosphere, oxalyl chloride (0.72 ml, 8.3 mmol) was added to a slurry of 1-(11-yl-undecanoic acid)-3,7-dimethylxanthine (2.0 g, 5.5 mmol), prepared in example 9 above, in dichloromethane (20 ml). The reaction was heated to reflux and allowed to stir for 1 hour. The resulting solution was cooled to ambient temperature and then slowly transferred to a stirring solution of 3,4,5-trimethoxybenzylamine (2.8 ml, 16.5 mmol) in dichloromethane (20 ml), followed by cooling to 0.degree. C. After 2 hours of stirring at ambient temperature, the reaction was poured into 3% aqueous hydrogen chloride solution (100 ml), followed by saturated aqueous salt solution (40 ml). The mixture was extracted with three 50 ml aliquots of dichloromethane. The combined organic layers were washed with saturated aqueous salt solution (50 ml) and dried over magnesium sulfate. The solvents were evaporated under reduced pressure, leaving a crude yellow residue. Column chromatography over alumina using an ethyl acetate/ ethyl acetate-methanol eluant and subsequent recrystallization from ethyl acetate produced 0.98 g of a white solid, inventive compound no. 3577 (33% yield).

EXAMPLE 13

This example shows an inhibitive effect of inventive compounds nos. 3549 and 3546 on murine thymocyte proliferation stimulated by Concanavalin A (ConA) and interleukin-2 (IL-2). This assay is an in vitro, predictive model of a compound's therapeutic potential in treating or preventing autoimmune, immune or inflammatory diseases. Procedurally, thymuses were obtained from normal, female Balb/C mice. The thymuses were

dissociated and plated into 96-well plates at a density of 2.times.10.sup.5 cells/well. ConA (0.25 mg/ml) and IL-2 (12.5 ng/ml) were added to the wells. Drug was added at various doses two hours prior to activation with ConA and IL-2. The cells were incubated for 4 days at 37.degree. C. On day 4, the cells were pulsed with tritiated thymidine and allowed to incubate for an additional 4 hours. Harvested cells were analyzed for incorporated tritiated thymidine, determined using a liquid scintillation counter. Dose response curves were prepared from the assay results and used to calculate an IC50 value for each compound tested.

In representative dose response curves prepared for assays investigating compounds nos. 3546 and 3549, FIGS. 1 and 2, respectively, illustrate the inhibitive effects of these compounds on proliferation of thymocytes stimulated with ConA and IL-2. Background counts, without addition of representative inventive compounds were about 190 cpm. FIG. 1 illustrates a remarkable ability of inventive compound no. 3546 to inhibit proliferation of thymocytes in this system. FIG. 2 illustrates a less pronounced ability of the inventive compounds to inhibit thymocyte proliferation, suggesting specificity of particular inventive compounds for treating specific diseases. As shown, inventive compound no. 3546 inhibited ConA/IL-2 stimulated proliferation at compound concentrations less than 20 .mu.M, with an IC50 value, experimentally calculated from this dose response curve, of about 4.8 .mu.M. These concentrations plotted are within concentrations known to be achieved in vitro for treating disease.

EXAMPLE 14

This example illustrates an ability of inventive compounds nos. 1514 and 1583 to inhibit proliferation of peripheral blood mononuclear cells (PBMC) in response to allogeneic stimulation. This in vitro mixed lymphocyte reaction (MLR) assay is useful in assessing biological activity of an inventive compound. Procedurally, PBMC were obtained by drawing whole blood from healthy volunteers in a heparinized container, the whole blood samples diluted with an equal volume of hanks balanced salt solution (HBSS).

This mixture was layered on a sucrose density gradient, such as a Ficoll-Hypaque.RTM. gradient (specific gravity 1.08), and centrifuged (1000.times.g) for 25 minutes at no warmer than room temperature. PBMC were obtained from a band at a plasma-Ficoll interface, separated and washed at least twice in a saline solution, such as HBSS. Contaminating red cells are lysed, for example, by ACK lysis for 10 minutes at 37.degree. C., and the PBMC were washed twice in HBSS. The pellet of purified PBMC was resuspended in complete medium, such as RPMI 1640 plus 20% human inactivated serum.

Proliferative response of PBMC to allogeneic stimulation was determined in a two-way MLR performed in a 96-well microtiter plate. Approximately 10.sup.5 test-purified PBMC in 200 .mu.l complete medium were co-cultured with approximately 10.sup.5 autologous (control culture) or allogeneic (stimulated culture) PBMC. Allogeneic cells were from HLA disparate individuals. Varying doses of compounds nos. 1514 and 1583 were added simultaneously upon addition of cells to the microtiter plate. The cultures were incubated for 6 days at 37.degree. C. in a 5% CO.sub.2 atmosphere, after which time, tritiated thymidine was added (for example, 1 .mu.Ci/well of 40 to 60 Ci/mmole) and proliferative inhibition was assessed by determining amount of tritiated thymidine taken up, using liquid scintillation counting.

FIGS. 3 and 4 are plotted graphs of compound concentrations (.mu.M) versus inhibition (as a function of incorporated thymidine, cpm) for compounds nos. 1514 and 1583, respectively. FIGS. 3 and 4 illustrate an ability of the inventive compounds tested to inhibit PBMC proliferation. At concentrations less than 250 .mu.M, compound no. 1583 more significantly inhibited incorporation of thymidine. Similarly, although to lesser degrees in comparison to compound no. 1583, compound no. 1514 inhibited proliferation in this MLR assay at compound concentrations less than 250 .mu.M.

EXAMPLE 15

This example illustrates inhibitive effects of the inventive compounds on Balb/3T3 cell proliferation in response to platelet derived growth factor (PDGF) stimulation.

Disregulated PDGF-proliferative response has been linked to a variety of diseases, including, e.g., restenosis, atherosclerosis, fibrosis, and tumor cell angiogenesis. Balb/3T3 cells respond vigorously to PDGF stimulation, and are useful in vitro models for further study of PDGF-induced proliferation. In an assay useful in determining whether a compound would be useful in treating diseases characterized by this or similar disregulated proliferative responses, research indicates that many of the inventive compounds inhibit PDGF-induced proliferation of Balb/3T3 cells.

Balb/3T3 cells were plated in low serum-containing medium for 24 hours prior to stimulation with various

concentrations of inventive compound. Specifically, in this assay, inventive compounds nos. 1529, 2538, 3537, 3542, 3546, 3554, 3557, 3559, 3562, 3564, 3571, 3573 and 3577 were tested. PDGF was added at varying concentrations along with tritiated thymidine. The cells were allowed to incubate for one day, following addition of PDGF and thymidine. 24 hours later, the cells were harvested and counted by liquid scintillation counting. Data obtained for each compound were plotted as % inhibition versus concentration of inventive compound and IC50 values experimentally calculated from the results plotted.

In conjunction with the Balb/3t3 proliferation assay, a related viability assay was conducted to assess the cytotoxicity of compounds which inhibit proliferation in this system. The assay protocol was identical to that performed above except that tritiated thymidine was not added after the 24 hour incubation with PDGF. Subsequent to incubation, a 10 .mu.M solution of 2,7-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein, acetoxymethyl ester (BCECF--a compound that when cleaved by esterases, yields a fluorescent product, thus providing a measure of cell number) was added and the cells incubated for 30 minutes at 37.degree. C. Following this incubation, BCECF was replaced with PBS and the plate read for fluorescence in a Millipore "cytofluor". Data obtained were plotted as a percent of control versus concentration of inventive compound tested and fifty percent (50%) lethal dose concentrations (LD50) for the inventive compounds tested were experimentally calculated from the plotted data.

FIG. 5 reports the experimentally calculated IC50 values obtained in the foregoing proliferation assay and LD50 values obtained in the corresponding viability assay for each inventive compound tested. The reported results indicate that many of the inventive compounds have IC50 values--the concentration of inventive compound in the proliferation assay inhibiting 50% proliferation of a control level--less than 10 .mu.M. Specifically, inventive compounds nos. 3554, 3559, 3571 and 3577 have IC50 values at or below 1 .mu.M. Of significance, compound no. 3577 inhibits 50% proliferation at an extremely low concentration of 0.1 .mu.M!

LD50 values reported in viability assays for the inventive compounds tested indicate that many of the compounds have LD50 values above measurable levels. In FIG. 5, experimentally calculated IC50 values which equaled or exceeded 20 .mu.M were reported as 20 .mu.M. For a majority of compounds tested, a significant concentration interval exists between the IC50 and LD50 experimentally calculated, indicating that the inventive compounds are not only candidates for treating or preventing restenosis, atherosclerosis, fibrosis, tumor cell angiogenesis and other similar diseases, but possess significant windows for therapeutic treatment.

* * * * *

United States Patent **6,103,730**
Klein , et al. [redacted]5,2000

Amine substituted compounds

Abstract

Compounds and pharmaceutical compositions, including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof, have the formula: CORE MOIETY--(R).sub.j In these compounds, j is an integer from one to three; the core moiety is a cyclic core, the cyclic core being non-cyclic or at least one five- to seven-member non-heterocyclic ring or heterocycle; and R is selected from the group consisting of amine, hydrogen, halogen, hydroxyl, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, cyclic or heterocyclic group or formula I. At least one R having formula I: ##STR1## In formula I, n is an integer from four to twenty; and each R.sub.1 or R.sub.2 is independently hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or cyclic or heterocyclic group. The compounds are useful in treating or preventing, for example, sepsis syndrome, hematopoietic or organ toxicity, cancer, viral activity, AIDS and AIDS-related indications, allopecia caused by cytotoxic therapies, and progression of an inflammatory or autoimmune disease.

Inventors: **Klein; J. Peter** (Vashon, WA); **Underiner; Gail E.** (Brier, WA); **Kumar; Anil M.** (Seattle, WA); **Ridgers; Lance H.** (Bothell, WA)
Assignee: [redacted]
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Primary Examine
Attorney, Agent or Firm

Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This is a Continuation in Part of U.S. application Ser. No. 08/217,051, filed Mar. 24, 1994 ABN.

Claims

What is claimed is:

1. A therapeutic compound, including resolved enantiomers, diastereomers, hydrates, salts, o[redacted] thereof, having the formula:

CORE MOIETY--(R).sub.j

wherein:

j is an integer from one to three;

the core moiety is xanthinyl; and

R is independently selected from the group consisting of amine, hydrogen, halogen, hydroxyl, C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, 2-bromopropyl, 4-chloropentyl, cyclohexyl, cyclopentyl, 3-dimethylaminobutyl, 2-hydroxyethyl, 5-hydroxyhexyl, 3-hydroxy-n-butyl, 3-hydroxypropyl, 2-methoxyethyl, 4-methoxy-n-butyl, phenyl and formula I, at least one R being bonded to a nitrogen at the one position of the core moiety and having formula I: ##STR6## wherein: (CH.sub.2).sub.n is optionally substituted by a C.sub.(1-10) alkyl or a C.sub.(2-10) alkenyl group that is optionally substituted by a member selected from the group consisting of C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl, C.sub.(1-8) alkoxyl, C.sub.(1-8) hydroxyalkyl, azido, carboxyl, cyano, C.sub.(1-8) haloalkyl, isocyano, mercaptocarbonyl, thioureido and ureido;

n is an integer from five to twenty; and

each R.sub.1 or R.sub.2 is independently hydrogen, optionally substituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl or C.sub.(2-20) alkenyl, carbocyclic group or heterocyclic group, with the proviso that at least one of R.sub.1 or R.sub.2 is other than hydrogen or methyl;

wherein the carbocyclic group or heterocyclic group is a member selected from the group consisting of bicyclo [4.4.0]decanyl, bicyclo[2.2.1]heptanyl, bicyclo[3.2.0]heptanyl, bicyclo[4.1.0]heptanyl, bicyclo[2.2.1]hexanyl, bicyclo[4.3.0]nonanyl, bicyclo[2.2.2]octanyl, biphenyl, cyclopentadienyl, cyclopentanyl, cyclobutanyl, cyclobutenyl, cycloheptanyl, cyclohexanyl, cyclooctanyl, cyclopropanyl, fluorenyl, indenyl, phenyl, quinonyl, terphenyl, napthalenyl, azetidinyl, benzofuranyl, benzothiophenyl, furanyl, glutarimidyl, indolyl, isoquinolinyl, oxazolyl, oxetanyl, oxiranyl, phthalimidyl, piperidinyl, pyrrolidinyl, pyranyl, pyridinyl, pyrrolyl, quinolinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydrothiophenyl, thiophenyl and thyminyl;

wherein, when the R.sub.1 or R.sub.2 is substituted and is other than the substituted C.sub.(1-20) alkyl, the substituent is selected from the group consisting of C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl, C.sub.(1-8) alkoxyl, C.sub.(1-8) hydroxyalkyl, azido, carboxyl, cyano, C.sub.(1-8) haloalkyl, aryl, halogen, oxo, isocyano, mercaptocarbonyl, thioureido and ureido, with the proviso that when R.sub.1 or R.sub.2 is a carbocyclic or heterocyclic group, then the substituent is not aryl, halogen or oxo; and

wherein when the R.sub.1 or R.sub.2 is a substituted C.sub.(1-20) alkyl, the substituent is selected from the group consisting of C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl, C.sub.(1-8) alkoxyl, C.sub.(1-8) hydroxyalkyl, azido, carboxyl, cyano, C.sub.(1-8) haloalkyl, aryl, halogen, oxo, isocyano, mercaptocarbonyl, thioureido and ureido.

2. A therapeutic compound, including resolved enantiomers, diastereomers, hydrates, salts, or solvates thereof, having the formula:

CORE MOIETY--(R).sub.j

wherein:

j is an integer from one to three;

the core moiety is xanthinyl; and

R is independently selected front the group consisting of amine, hydrogen, halogen, hydroxyl, C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, 2-bromopropyl, 4-chloropentyl, cyclohexyl, cyclopentyl, 3-dimethylaminobutyl, 2-

hydroxyethyl, 5-hydroxyhexyl, 3-hydroxy-n-butyl, 3-hydroxypropyl, 2-methoxyethyl, 4-methoxy-n-butyl, phenyl and formula I, at least one R being bonded to a nitrogen at the one position of the core moiety and having formula I: ##STR7## wherein: (CH.sub.2).sub.n is optionally substituted by a C.sub.(1-10) alkyl or a C.sub.(2-10) alkenyl group that is optionally substituted by a member selected from the group consisting of C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl, C.sub.(1-8) alkoxyl, C.sub.(1-8) hydroxyalkyl, azido, carboxyl, cyano, C.sub.(1-8) haloalkyl, isocyano, mercaptocarbonyl, thioureido and ureido;

n is an integer from five to twenty; and

one of R.sub.1 or R.sub.2 is hydrogen and the other of R.sub.1 or R.sub.2 is an unsubstituted C.sub.(2-20) alkyl or a C.sub.(1-20) substituted alkyl substituted by an unsubstituted heterocyclic group selected from the group consisting of indenyl quinonyl, azetidinyl, benzofuranyl, furanyl, glutarimidyl, indolyl, isoquinolinyl, oxazolyl, phthalimidyl, piperidinyl, pyrrolidinyl, pyranyl, pyridinyl, pyrrolyl, quinolinyl, tetrahydrofuranyl, tetrahydropyranyl, and thyminyl.

3. A compound selected from the group consisting of: ##STR8##

4. A pharmaceutical composition comprising a compound according to claim 1 and a suitable carrier, diluent or excipient.

5. The pharmaceutical composition of claim 4, wherein the composition is formulated for parenteral, topical or oral administration or for inhalation.

6. The compound according to claim 1, wherein n is an integer from five to sixteen.

7. The compound according to claim 1, wherein n is an integer from seven to fourteen.

Description

TECHNICAL FIELD OF THE INVENTION

The invention provides for a class of amine-substituted compounds that are effective agents for inhibiting specific cellular signaling events often induced by noxious or inflammatory stimuli, or to be anti-microbial to yeast or fungal infections, directly or indirectly (i.e., immune stimulation). More specifically, the inventive compounds have at least one amine-containing substituent bonded to a core moiety. The inventive compounds are useful antagonists for controlling intracellular levels of specific non-arachidonyl sn-2 unsaturated phosphatidic acids and corresponding phosphatidic acid-derived diacylglycerols, which occur in response to cellular proliferative stimuli. In addition, the compounds are useful in treating or preventing, for example, sepsis syndrome, hematopoietic or organ toxicity, cancer, viral activity, AIDS and AIDS-related indications, allopecia caused by cytotoxic therapies, and progression of an inflammatory or autoimmune disease.

BACKGROUND OF THE INVENTION

Pentoxifylline [1-(5-oxohexyl)-3,7-dimethylxanthine], abbreviated PTX, is a xanthine derivative widely used medically for increasing blood flow. U.S. Pat. Nos. 3,422,107 and 3,737,433, both to Mohler et al. disclose PTX. Metabolites of PTX were summarized in Davis et al., "Microbial Models of Mammalian Metabolism: Microbial Reduction and oxidation of Pentoxifylline," Applied and Environmental Microbiology, Vol. 48, No. 2, pages 327-381, August 1984, and Bryce et al., "Metabolism and Pharmacokinetics of .sup.14 C-Pentoxifylline in Healthy Volunteers," Arzneim.-Forsch./Drug Res. Vol. 39, No. 4, pages 512-517, 1989. A metabolite of PTX is 1-(5-hydroxyhexyl)-3,7-dimethylxanthine, designated M1. M1 was also disclosed as increasing cerebral blood flow in U.S. Pat. Nos. 4,515,795 and 4,576,947 to Hinze et al. Other metabolites include 1-(5-pentyl)-3,7-dimethylxanthine carboxylic acid, designated M4, and 1-(4-butyl)-3,7-dimethylxanthine carboxylic acid, designated M5. In addition, U.S. Pat. Nos. 4,833,146 and 5,039,666 to Gebert et al. and Novick, respectively, disclose use of tertiary alcohol analogs of xanthine for enhancing cerebral blood flow.

PTX and its known metabolites thereof have been shown to have in vivo activity in specific biologic systems. U.S. Pat. No. 4,636,507 to Kreutzer et al. describes an ability of PTX and M1 to enhance chemotaxis in polymorphonuclear leukocytes responding to chemotaxis stimulation. In addition, PTX and related tertiary alcohol substituted xanthines inhibit activity of certain cytokines to affect chemotaxis as described in U.S. Pat.

Nos. 4,965,271 and 5,096,906 to Mandell et al. Furthermore, by co-administrating PTX and GM-CSF, patients undergoing allogeneic bone marrow transplant exhibited decreased levels of tumor necrosis factor, TNF. Bianco et al., "Pentoxifylline (PTX) and GM-CSF Decrease Tumor Necrosis Factor (TNF-.alpha.) Levels in patients undergoing allogeneic Bone Marrow Transplantation (MBT)," Blood, Vol. 76, No. 1, Suppl. 1 (522), page 133a, 1990. Reduction in assayable levels of TNF was accompanied by reduced BMT-related complications. However, in normal volunteers, TNF levels were higher among PTX recipients. Therefore, elevated levels of TNF are not the primary cause of such complications.

Further research with PTX, its metabolites and their activity relating to various biologic systems spurred investigations with potential therapeutic agents heretofore unknown. These agents were identified as potential therapies for treating or preventing disease by inhibiting secondary cellular response to an external or in situ primary stimuli. These investigations sought efficacious therapeutic compounds which would be safe and effective for human or animal administration and would maintain cellular homeostasis in the presence of a variety of inflammatory stimuli.

Many diseases are difficult to treat because they have complex mechanisms of action, and multiple, adverse effects on a subject. As an example, cancer has been difficult to treat for this and other reasons. Precise causes of cancer remain unknown. Malignant tumor growth results from many physiologic factors. Cancer cells metastasize (i.e., break through blood vessels and travel to distant body sites) and secrete enzymes called metalloproteases, which "break down" blood vessel walls, allowing the cancer cells to enter the bloodstream and form remote tumors (proteolysis). In addition, tumor cell adhesion receptors (integrins) effect attachment--necessary for tumor residence in organs--of tumor cells to blood vessel walls and normal organs. Cancer cells also secrete certain proteins, such as BFGF, that stimulate new blood vessel development (angiogenesis), these new blood vessels supplying nutrients to promote malignant tumor growth.

Conventional antineoplastic therapies, such as, for example, antimetabolites, alkylating agents and antitumor agents (which target or interfere with DNA and/or synthesis of DNA or its precursors), and biologic therapies (including selective interferons, interleukins and other factors) have significant adverse side effects in patients, not limited to acute toxicity due to effects on rapid-proliferating tissues, such as bone marrow and oral epithelium, myelosuppression and mucositis, renal failure and neurological, hepatic or pulmonary toxicity. Thus, for example, a cancer therapy which effectively prevented, reduced or eliminated malignant tumors without causing deleterious side effects would provide previously unknown treatment.

Searching for potential disease treatments which would prevent or treat a disease with minimal or no adverse side effects, compounds were discovered having biologic activity in multifarious, predictive assays, indicating potential therapy in treating a broad spectrum of clinical indications acting via a variety of disease mechanisms. However, all these mechanisms appear to affect the second messenger pathway. Results of this research are the subject matter of this disclosure, the compounds discussed herein having structures and remarkable and surprising properties heretofore unknown.

SUMMARY OF THE INVENTION

The invention provides amine-substituted compounds and pharmaceutical compositions and uses thereof. The inventive amine-substituted compounds are useful in a large variety of therapeutic indications for treating or preventing disease. In particular, the inventive compounds and pharmaceutical compositions thereof provide therapy for diseases caused or advanced by intracellular signaling through specific intracellular signaling pathways, specifically the pathways discussed herein, by inhibiting a proliferative signaling pathway. Abnormally-induced intracellular signaling is characteristic of diseases treatable using the inventive compounds.

The inventive compounds have the formula:

CORE MOIETY--(R).sub.j

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates or mixtures thereof. In this formula, j is an integer from one to three, the core moiety is non-cyclic or cyclic and R may be an amine, hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, a carbocyclic or heterocyclic group or formula I: ##STR2##

The inventive compounds have at least one R of formula I. In formula I, n is an integer from four to twenty, preferably 5 to sixteen, more preferably seven to fourteen; each R.sub.1 or R.sub.2 is independently hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or

carbocyclic or heterocyclic group, the alkyl or alkenyl being preferably substituted by an aryl, halogen or ketone group. Preferably, n is an integer from four to fourteen and more preferably n is an integer from six to ten. Optionally, (CH.sub.2).sub.n may 1) be substituted by a substituted or unsubstituted C.sub.(1-10) alkyl or C.sub.(2-10) alkenyl group; or 2) have one or two unsaturated bonds (preferably in a cis configuration). In most preferred compounds of the invention, R.sub.1 and R.sub.2 are both hydrogen or methyl or one of R.sub.1 or R.sub.2 is hydrogen and the other of R.sub.1 or R.sub.2 is an unsubstituted C.sub.(1-20) alkyl or a C.sub.(1-20) alkyl substituted by an unsubstituted heterocycle.

The inventive compounds are active therapeutic agents by virtue of an ability to prevent a second messenger signal from effecting an undesirable cell response. The core moiety serves as an orienting or plasma membrane-anchoring moiety. The orienting moiety may spacially orient the (R) structural component(s) of the inventive compounds, having the appropriately-substituted amine functional group, to an active site of an enzyme involved in phospholipid-based second messenger cellular signaling. Therefore, a large number of core moieties are active by virtue of their ability to orient a compound in a cellular plasma membrane.

A non-cyclic core moiety may include, but is not limited to, for example, acetamide, amide, amine, amino acid (one or two), carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol, thioester or the like. Exemplary core moiety amino acids may include, but are not limited to, one or more of the following: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The non-cyclic core moiety may preferably be an amide, carboxyl ester, carboxide, hydrogen, hydroxide or a dipeptide comprising two amino acids selected from the foregoing exemplary list. A non-cyclic, halogen-core moiety may be, for example, bromine, chlorine, fluorine or iodine.

A cyclic core may be at least one five- to seven-member, non-heterocyclic (i.e., carbocyclic) ring or a heterocycle. The at least one five- to seven-membered cyclic core may preferably have from one to three, five- to six-membered ring structures in a predominantly planar configuration. An exemplary, non-heterocyclic ring core moiety may be selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione; cyclopentanedione; napthlalene; phenol; quinone; salicylic acid; stilbene and tricyclododecane.

Although other heterocyclic cores are within the scope of the invention, the following representative cores are preferred: substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; purine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic; quinolizinedione; quinazolinone; quinoline; resorcinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K; or xanthine.

Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimide, methylthymine, thymine, uracil or xanthine core. In representative, preferred compounds, R of formula I may be bonded to an N.sub.1 nitrogen of glutarimide; N.sub.1 nitrogen of xanthine (and N.sub.3 and N.sub.7 xanthine nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, C.sub.(1-6) alkyl, fluoro, chloro and amino); N.sub.3 nitrogen of methylthymine; or N.sub.1 nitrogen of uracil. Alternatively, R having formula I may be bonded to N.sub.1 and N.sub.3 xanthine nitrogens and the N.sub.7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino.

The invention also provides a pharmaceutical composition. Pharmaceutical compositions of the inventive compounds comprise a pharmaceutical carrier or diluent and an effective amount of an inventive compound. Of course, the nature of the composition and the pharmaceutical carrier or diluent will depend upon the intended route of administration, for example, parenterally, topically, orally or by inhalation.

The invention includes a method for treating an individual having a variety of diseases. The disease is characterized by or can be treated by inhibiting an immune or cellular response to external or in situ primary stimuli. The disease states are mediated by a cellular response wherein intracellular signaling is mediated through a specific phospholipid-based second messenger pathway functioning within the cell and whose enzymes are located primarily, although not exclusively, near an inner leaflet of a cell plasma membrane. Various noxious or proliferative stimuli activate the second messenger pathway. Moreover, specific cytokines signal through specific phosphatidic acid intermediates, differentiated by the nature of fatty acids. This activation of the second messenger pathway is characteristic of disease states treatable using the inventive compounds or pharmaceutical compositions thereof. A biochemistry of this second messenger pathway is

described herein. Thus, more specifically, the invention provides methods for treating or preventing clinical symptoms of various disease states or reducing toxicity of other treatments by inhibiting cellular signaling through a second messenger pathway involving signaling through phosphatidic acid.

A disease state or treatment-induced toxicity, treatable with an inventive compound or pharmaceutical, composition thereof, include, but are not limited to, for example: tumor progression involving tumor stimulation of blood supply (angiogenesis) by production of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF); tumor invasion and formation of metastases through adhesion molecule binding, expressed by vascular endothelial cells (VCAM and ICAM); tissue invasion through tumor metalloprotease production such as MMP-9; autoimmune diseases caused by T cell or B cell immune system dysregulation (treatable by suppression of the T cell or B cell responses); acute allergic reactions including, but not limited to, asthma and chronic inflammatory diseases--mediated by proinflammatory cytokines including (TNF) and interleukin-l (IL-1)--and rheumatoid arthritis, osteoarthritis, multiple sclerosis or insulin-dependent diabetes mellitus (IDDM)--associated with enhanced localization of inflammatory cells and release of inflammatory cytokines and metalloproteases; smooth muscle cell, endothelial cell, fibroblast and other cell-type proliferation in response to growth factors, such as PDGF, FGF, endothelial growth factor (EGF), etc. (i.e., atherosclerosis, restenosis, stroke, and coronary artery disease); activation of human immunodeficiency virus infection (AIDS and AIDS related complex); HIV-associated dementia; kidney mesangial cell proliferation in response to IL-1, MIP-1.alpha., PDGF or FGF; kidney glomerular or tubular toxicity in response to cyclosporin A or amphotericin B treatment; organ toxicity (e.g., gastrointestinal or pulmonary epithelial) in response to a cytotoxic therapy (e.g., cytotoxic drug or radiation); inflammation, particularly when in response to inflammatory stimuli (e.g., TNF, IL-1 and the like) or characterized by production of metalloproteases or allergies due to degranulation of mast cells and basophils in response to IgE or RANTES; bone diseases caused by overproduction of osteoclast-activating factor (OAF) by osteoclasts; central nervous system disorders (CNS) diseases resulting from over-stimulation by pro-inflammatory neurotransmitters such as; acetylcholine, serotonin, leuenkephalin or glutamate; acute inflammatory diseases such as septic shock and adult respiratory distress syndrome; multi-organ dysfunction associated with inflammatory cytokine cascade; and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an effect of inventive compound no. 3506 on proliferation of human stromal cells stimulated by PDGF.

FIG. 2 illustrates inhibitive effects of inventive compound no. 3556 on proliferation of Balb/3T3 cells, stimulated with PDGF.

FIG. 3 reports cytotoxicity data obtained in the PDGF assay used to obtain data reported in FIG. 1 for compound no. 3556.

FIG. 4 illustrates inhibitive effects of inventive compound no. 4500 on proliferation of Balb/3T3 cells, stimulated with PDGF.

FIG. 5 reports cytotoxicity data obtained in the PDGF assay used to obtain data reported in FIG. 4 for compound no. 4500.

FIGS. 6, 7, 8, 9, 10 and 11 report results obtained for inventive compounds nos. 3506, 3556, 3563, 3576, 3581, 3582 and 3584 in a thymocyte proliferation assay in which thymocytes were co-stimulated with Con A and IL-2 and proliferation of thymocytes was measured.

FIGS. 12, 13, 14, 15 and 16 illustrate data obtained for inventive compounds nos. 3563, 3576, 3581, 3584 and 4500, respectively, in a cancer screening assay used to evaluate whether the inventive compounds, potential cancer therapies, have cytotoxic effects on normal human bone marrow cells.

FIGS. 17, 18, 19, 20 and 21 illustrate results obtained for inventive compounds nos. 3556, 3563, 3576, 3580, 3581, 3582, 3584, 3590, 3593, 4500, 4507 and 4508 in an assay measuring anti-viral activity of the inventive compounds for inhibiting gene expression directed by specific viral promoters in cell lines.

FIG. 22 illustrates an ability of the inventive compounds, as represented by inventive compound no.3556, to prevent promotion of JR-CSF strain of HIV-1 infection of human peripheral blood lymphocytes (PBL).

FIG. 23 shows the effects of two comparative compounds on PDGF-induced proliferation in human stromal

cells.

DETAILED DESCRIPTION OF THE INVENTION

The inventive compounds may control cell behavior by a particular phase of a second messenger pathway system (Bursten et al., "Interleukin-1 Rapidly Stimulates Lysophosphatidate Acyltransferase and Phosphatidate Phosphohydrolase Activities in Human Mesangial Cells," J. Biol. Chem. Vol. 266, No. 31, pages 20732-20743, Nov. 5, 1991). The second messengers are lipids or phospholipids and proteins and use the following abbreviations:

PE=phosphatidyl ethanolamine

LPE=lysophosphoethanolamine

PA=phosphatidic acid

LPA=lysophosphatidic acid

DAG=diacylglycerol

LPLD=lysophospholipase-D

LPAAT=lysophosphatidic acid acyl transferase

PAPH=phosphatidic acid phosphohydrolase

PLA.sub.2 =phospholipase A.sub.2

PLD=phospholipase D

PAA=phosphoarachidonic acid

PC=phosphatidyl choline

"remodeled" PA, cyclic pathway=PAA, LPA, PA and DAG intermediates substituted with 1-saturated, 2-linoleoyl or 1,2-dioleoyl, dioleoyl/1,2-sn-dilinoleoyl at the indicated sn-1 and sn-2 positions.

"Classical PI Pathway"=PI, DAG, PA intermediates substituted with 1-stearoyl, 2-arachidonoyl fatty acyl side chains.

"PLD-generated PA"=PE, PC, LPA, PA and DAG intermediates substituted with, e.g., 1,2-sn-dioleoyl-, 1-alkyl, 2-linoleoyl-, and 1-alkyl, 2-docosahexaneoyl-side chains.

Lysophosphatidic acid transferase (LPAAT) effects the synthesis of phosphatidic acid (PA) from lysophosphatidic acid (LPA) by incorporation of an acyl group from acyl CoA. Hydrolysis of the phosphate moiety by PA phosphohydrolase (PAPH) results in the formation of DAG. These aspects of the pathway appear to be activated immediately (within a minute) upon stimulation by a primary stimulus (e.g., a cytokine such as IL-1, IL-2 or TNF) acting at a receptor on a cellular surface. An immediate detectable effect is an elevation of levels of PA and DAG. The inventive compounds reduce or eliminate elevated PA and DAG.

These inventive compounds and pharmaceutical compositions are capable of, among other things, inhibiting subspecies of LPAAT and PAPH enzymes with substrate specificity for intermediates with 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. PTX also blocks PAPH in-a specific activation pathway that does not involve PI but rather derives from a PA that is largely composed of 1,2-diunsaturated and 1-alkyl, 2-unsaturated subspecies. This was shown, for example, by the demonstration that TNF-stimulated human mesangial cells produce DAG from PI and regenerate PI with or without PTX present. In the latter system there is no evidence to suggest that PA or DAG are derived from sources other than PI. In contrast, the inventive compounds affect that subset of PAPH and LPAAT relating to substrates with unsaturated fatty acids other than arachidonate in the sn-2 position, not the housekeeping forms of these enzymes that serve the PI pathway.

The second messenger pathway of most significance in the invention involves substrates with unsaturated

fatty acids in the sn-2 positi n other than arachidonate and those sub-species of PAPH and LPAAT and are not involved in normal cellular housekeeping functions, which are part of a classical PI pathway. The PAPH and LPAAT enzymes involved in this specific second messenger pathway are stereo-specific for different acyl side chains and substrate isomers. Therefore, the inventive compounds may preferably be substantially enantiomerically pure.

IL-1 activates (through the Type I IL-1 receptor) a lyso-PA acyltransferase (LPAAT) and phosphatidate phosphohydrolase (PAPH) within 5 seconds of cell (for example, human mesangial cells, HMC) exposure. Activation of both enzymes results in production of PA species with sn-1 and sn-2 unsaturated acyl groups, with the majority of sn-2 acyl chains being polyunsaturated. Both IL-1 and a product of LPAAT (1,2-sn-dilinoleoyl PA) activate a signaling pathway involving hydrolysis of PE to PA. This reaction is followed by dephosphorylation of PA to produce both 1,2-sn-diacylglycerol and 1-o-alkyl, or 1-o-alkenyl, acylglycerol (AAG) species. The inventive compounds exert their activity by inhibiting one or both enzymes at an inner leaflet of the plasma membrane. Therefore, appropriate in vitro models for drug activity may measure inhibition of stimulation caused by a proinflammatory cytokine or other inflammatory cellular signal.

Generation of sn-2 unsaturated PA fraction by LPAAT serves to activate either G-proteins, or acts directly upon PLD through alteration of its lipid microenvironment. Activation of LPAAT and generation of the sn-2-unsaturated PA species is an energy sensitive pathway of PLD. This provides a mechanism for a limited-receptor system to amplify a signal and generate a cellular response by rapid synthesis of small amounts of PA. Uptake of di-unsaturated PA, which is less than about 0.1% of total membrane lipid mass, is sufficient to activate PLD activity. This quantity of PA is similar to that endogenously synthesized by LPAAT. The PA-stimulated PLD acts upon PE, should localize to the inner leaflet of the cell membrane, enriched in PE relative to the outer leaflet. Therefore, the cellular inflammatory response to IL-1 is mediated by the pathway: IL-1R.fwdarw.PA.fwdarw.(PLD).fwdarw.PE. Whereas a localized tissue response is: lysoPA.fwdarw.PI.fwdarw.PKC.fwdarw.(PLD).fwdarw.PC. The PLD species are different isozymes. The second messenger pathway whose activation is inhibited by the inventive compounds is not a PI-derived pathway and does not involve PKC in the time courses of inhibition. PKC is acutely activated by PI-derived DAG, but chronic activation (i.e., >30 minutes) is maintained by PC-derived PA generated by PC-directed PLD. Therefore, the pathway inhibited by the inventive compounds is PE-directed and not PC-directed. Moreover, the PE-directed PLD favors substrates with sn-2 long-chain unsaturation.

DAG and PA are upregulated in oncogenically transformed cells. For example, activating ras mutations result in increased generation of DAG upon stimulation with mitogens, although the sources of DAG differ between experimental systems. In nontransformed renal mesangial cells, IL-1.beta. stimulation increased PLA.sub.2 and LPAAT activation, resulting in generation of sn-2 unsaturated PA and subsequent hydrolysis to DAG by phosphatidate phosphohydrolase. The ras transformation in NIH/3T3 cells upregulates serum-stimulated generation of DAG and PA. A particular specie of serum-stimulated DAG is dioleoyl and of PA are dilinoleoyl and dioleoyl. This upregulation occurs over 4-12 hours and pretreatment of cells with an inventive compound, blocks generation of these phospholipid second messengers. The inhibition occurs either through suppressing PA generation de novo from lysoPA, or through inhibition of one or both arms of the Lands cycle. A corresponding lysoPA increase with diminished PA/DAG production suggests inhibition of transacylation of a precursor lipid. Therefore, the ras transformation mediates an upregulation of PA through indirect stimulation of PLA.sub.2 and/or LPAAT activity. The inventive compounds inhibit conversion of upregulated lysoPA to PA and subsequently block phenotypic changes induced by PA/DAG in the membrane.

Therapeutic Uses of the Inventive Compounds

Inhibition of second messenger pathway activation, as described above, predicts that the inventive compounds are useful in treating a wide variety of clinical indications mediated at the cellular level by a common mechanism. Moreover, in vitro data presented herein provides predictive evidence that a wide variety of clinical indications, having similar effects on the selective second messenger pathway, may be treated by the inventive compounds. These compounds specifically inhibit the second messenger signaling pathway described above. In fact, the mechanism of action of the inventive compounds explains why these compounds have multifarious applications in treating a broad variety of clinical indications.

Activation of the second-messenger pathway is a significant mediator of response to noxious stimuli and results in cellular signals that lead to, for example, acute and chronic inflammation, immune response and cancer cell growth. Although the inventive compounds may desirably inhibit other noxious stimuli not discussed, they most effectively mediate the above conditions. Signals mediated by the present second messenger pathway include, for example, those cellular responses of lipopolysaccharide (LPS) directly; T cell activation by antigen; B cell activation by antigen, cellular responses to IL-1 (mediated through the IL-1 Type

I receptor but not the IL-1 Type II receptor) and TNF (Type I receptor), growth stimulated by transformations including, but not limited to, activated oncogenes (e.g., ras, abl, her 2-neu and the like), smooth muscle cell proliferation stimulated by PDGF, b-FGF and IL-1; T cell and B cell growth stimulation by IL-2, IL-4 or IL-7 and IL-4 or IL-6, respectively; and more generally, T cell receptor signaling.

Several compounds are particularly useful as inhibitors of IL-2-induced proliferative responses. Inhibiting IL-2 signaling is potentially useful in treating numerous diseases characterized by T-cell activation and hyperproliferation. Representative autoimmune diseases treated by inhibiting IL-2 signaling include, but are not limited to, lupus, scleroderma, rheumatoid arthritis, multiple sclerosis, glomerula nephritis as well as potential malignancies, such as, for example, chronic myelogenous leukemia as well as others.

The inventive compounds: (1) block IL-1 signal transduction through the Type 1 receptor as shown, for example, by preventing IL-1 and IL-1 plus PDGF-induced smooth muscle, endothelial and kidney mesangial cell proliferation; (2) suppress up-regulation of adhesion molecules as shown, for example, by blocking VCAM in endothelial cells; (3) inhibit TNF-, LPS- and IL-1-induced metalloproteases (an inflammation model); (4) block LPS-, TNF- or IL-1-induced metalloprotease and secondary cytokine production (modeling prevention or treatment of septic shock); (5) suppress T cell and B cell activation by antigen, for example, IL-2 and IL-4; (6) inhibit mast cell activation by immunoglobulin E (IgE); (7) are cytotoxic for transformed cells and tumor cell lines, yet not for normal cells; and (8) block signaling by IL-2, IL-4, IL-6 and IL-7 on T and B cells.

The foregoing molecular and cellular effects give rise to the following therapeutic and pharmacologic effects, including, but not limited to: protection and treatment of endotoxic shock and sepsis induced by gram positive or gram negative bacteria; inhibition of tumor cell growth; synergistic immunosuppression active in autoimmune diseases and in suppressing allograft reactions; and stimulation of hair grow through reversal of an apoptotic process. The inventive compounds are most potent when used to prevent and/or treat septic shock, acute and chronic inflammatory disease, cancer growth and an autoimmune disease.

The inventive compounds also are useful as an adjuvant to inhibit toxic drug side effects (i.e., Il-2, amphotericin B and cytoreductive therapies) mediated through the second messenger pathway. Furthermore, the compounds of the invention are able to decrease enhanced levels of a relevant PA and DAG resulting from stimulation of synaptosomes with acetylcholine and/or epinephrine. This predicts that the effects of the compounds of the invention are to both enhance the release of inhibitory neural transmitters such as dopamine, and to modulate the distal "slow current" effects of such neurotransmitters.

Metalloproteases mediate tissue damage such as glomerular diseases of the kidney, joint destruction in arthritis, and lung destruction in emphysema, and play a role in tumor metastases. Three examples of metalloproteases include a 92 kD type V gelatinase induced by TNF, IL-1 and PDGF plus BFGF, a 72 Kd type IV collagenase that is usually constitutive and induced by TNF or IL-1, and a stromelysin/PUMP-1 induced by TNF and IL-1. The inventive compounds can inhibit TNF or IL-1 induction of the 92 kD type V gelatinase inducable metalloprotease. Moreover, the inventive compounds can reduce PUMP-1 activity induced by 100 U/ml of IL-1. Accordingly, the inventive compounds prevent induction of certain metalloproteases induced by IL-1 or TNF and are not involved with constitutively produced proteases (e.g., 72 Kd type IV collagenase) involved in normal tissue remodeling.

The inventive compounds inhibit signal transduction mediated through the Type I IL-1 receptor, and are therefore considered as IL-1 antagonists. A review article described the role of IL-1 as "an important rapid and direct determinant of disease. In septic shock, for example, IL-1 acts directly on the blood vessels to induce vasodilatation through the rapid production of platelet activating factor and nitric oxide, whereas in autoimmune disease it acts by stimulating other cells to produce cytokines or enzymes that then act on the target tissue." Dinarello et al., "The Role of Interleukin-1 in Disease," N. Engl. J. Med. Vol. 328, page 106, Jan. 14, 1993. The article describes a group of diseases that are mediated by IL-1, including sepsis syndrome, rheumatoid arthritis, inflammatory bowel disease, acute and myelogenous leukemia, IDDM, atherosclerosis and other diseases including transplant rejection, graft versus host disease (GVHD), psoriasis, asthma, osteoporosis, periodontal disease, autoimmune thyroiditis, alcoholic hepatitis, premature labor secondary to uterine infection and even sleep disorders. Since the inventive compounds inhibit cellular signaling through the IL-1 Type I receptor and are IL-1 antagonists, the inventive compounds are useful for treating all of the above-mentioned diseases.

For example, for sepsis syndrome, the mechanism of IL-1-induced shock appears to be the ability of IL-1 to increase the plasma concentrations of small mediator molecules such as platelet activating factor, prostaglandin and nitric oxide. These substances are potent vasodilators and induce shock in laboratory animals. Blocking the action of IL-1 prevents the synthesis and release of these mediators. In animals, a

single intravenous injection of IL-1 decreases mean arterial pressure, lowers systemic vascular resistance, and induces leukopenia and thrombocytopenia. In humans, the intravenous administration of IL-1 also rapidly decreases blood pressure and doses of 300 ng or more per kilogram of body weight may cause severe hypotension. The therapeutic advantage of blocking the action of IL-1 resides in preventing its deleterious biological effects without interfering with the production of molecules that have a role in homeostasis. The present inventive compounds address this need, identified by Dinarello et al., by inhibiting cellular signaling only through the IL-1 Type I receptor and not through the IL-1 Type II receptor.

With regard to rheumatoid arthritis, Dinarello et al. state: "Interleukin-1 is present in synovial lining and synovial fluid of patients with rheumatoid arthritis, and explants of synovial tissue from such patients produce IL-1 in vitro. Intraarticular injections of interleukin-I induce leukocyte infiltration, cartilage breakdown, and periarticular bone remodeling in animals. In isolated cartilage and bone cells in vitro, interleukin-1 triggers the expression of genes for collagenases as well as phospholipases and cyclooxygenase, and blocking its action reduces bacterial-cell-wall-induced arthritis in rats." Therefore, the inventive compounds, as IL-1 antagonists, are useful to treat and prevent rheumatoid arthritis.

With regard to inflammatory bowel disease, ulcerative colitis and Crohn's disease are characterized by infiltrative lesions of the bowel that contain activated neutrophils and macrophages. IL-1 can stimulate production of inflammatory eicosanoids such as prostaglandin E.sub.2 (PGE.sub.2), leukotriene B.sub.4 (LTB.sub.4) and IL-8, an inflammatory cytokine with neutrophil-chemoattractant and neutrophil-stimulating properties. Tissue concentrations of PGE.sub.2 and LTB.sub.4 correlate to severity of disease in patients with ulcerative colitis. Patients with inflammatory bowel disease have high tissue concentrations of IL-1 and IL-8. Therefore, an IL-1 antagonist, such as the inventive compounds, are effective to treat inflammatory bowel disease.

With regard to acute and chronic myelogenous leukemia, there is increasing evidence that IL-1 acts as a growth factor for such tumor cells. Therefore, the inventive compounds are effective to prevent the growth of worsening of disease for acute and chronic myelogenous leukemias.

IDDM is considered to be an autoimmune disease destroying beta cells in the islets of Langerhans, mediated by immunocompetent cells. Islets of animals with spontaneously occurring IDDM (e.g., BB rats or NOD mice) have inflammatory cells that contain IL-1. Therefore, the inventive compounds are useful for preventing and treating IDDM.

IL-1 also plays a role in atherosclerosis development. Endothelial cells are a target of IL-1. IL-1 stimulates proliferation of vascular smooth muscle cells. Foam cells, isolated from fatty arterial plaques from hypercholesterolemic rabbits, contain IL-1.beta. and IL-1.beta. messenger RNA. The uptake of peripheral blood monocytes results in initiation of IL-1 production by these cells. IL-1 also stimulates production of PDGF. Taken together, IL-1 plays a part in the development of atherosclerotic lesions. Therefore, an IL-1 antagonist, such as the inventive compounds are useful in preventing and treating atherosclerosis.

Excessive or unregulated TNF (tumor necrosis factor) production is implicated in mediating or exacerbating a number of diseases including rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfision injury, graft versus host reaction, allograft rejections, fever, myalgias due to infection such as influenza, cachexia secondary to infection, AIDS or malignancy, other viral infections (e.g., CMV, influenza, adenovirus, herpes family), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis, or pyresis. The inventive compounds or pharmaceutically acceptable salts thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of any disease state in a human or other mammal, which is exacerbated or signaled through the selective second messenger cellular phospholipid-based signaling pathway and by excessive or unregulated production of "first messenger" inflammatory cytokines such as TNF or IL-1. With regard to TNF primary stimuli, there are several disease states in which excessive or unregulated TNF production by monocytes/macrophages is implicated in exacerbating or causing the disease. These include, but are not limited to, for example, neurodegenerative diseases such as Alzheimer's disease, endotoxemia or toxic shock syndrome (Tracey et al., "Anti-cachectin/TNF Monoclonal Antibodies Prevent Septic Shock During Lethal Bacteraemia," Nature, Vol. 330, pages 662-664, 1987 and Hinshaw et al., "Survival of Primates in LD.sub.100 Septic Shock Following Therapy With Antibody to Tumor Necrosis Factor (TNF)," Circ. Shock, Vol. 30, pages 279-292, 1990); cachexia (Dezube et al., "Pentoxifylline and Wellbeing in Patients with Cancer," The Lancet, page 662, 1990), and adult respiratory distress syndrome (Millar et al., "Tumour Necrosis Factor in Bronchopulmonary Secretions of Patients with Adult Respiratory Distress Syndrome," The Lancet, Vol. 1,

pages 712-713, 1989). The inventive compounds may be used topically in the treatment of prophylaxis of topical disease states mediated or exacerbated by excessive TNF or IL-1, such as viral infections (herpes or viral conjunctivitis), psoriasis, fungal or yeast infections (ringworm, athletes foot, vaginitis, dandruff, etc.) or other dermatologic hyperproliferative disorders. High TNF levels have been implicated in acute malaria attacks (Grau et al., "Tumor Necrosis Factor and Disease Severity in Children with Falciparum Malaria," N. Engl. J. Med. Vol. 320, No. 24, pages 1586-1591, 1989), chronic pulmonary inflammatory diseases such as silicosis and asbestosis (Piguet et al., "Requirement of Tumour Necrosis Factor for Development of Silica-induced Pulmonary Fibrosis," Nature, Vol. 344, pages 245-247, 1990, and Bissonnette et al., "Pulmonary Inflammation and Fibrosis in a Murine Model of Asbestosis and Silicosis," Inflammation, Vol. 13, No. 3, pages 329-339, 1989), and reperfusion injury (Vedder et al., "Inhibition of Leukocyte Adherence by Anti-CD18 Monoclonal Antibody Attenuates Reperfusion Injury in the Rabbit Ear, Proc. Natl. Acad. Sci. USA, Vol. 87, pages 2643-2646, 1990).

The inventive compounds provide a method for maintaining homeostasis in cells contacted by primary stimuli by mitigating the effects of these primary stimuli on the secondary signaling pathways invoked within seconds of a primary stimulus. For example, administration of an inventive compound in vivo or ex vivo provides a method to modify cellular behavior, the method comprising contacting cells (in vivo or ex vivo), whose behavior is to be modified, with an effective amount of an inventive compound or a pharmaceutical composition thereof wherein said method is a method to: (1) inhibit proliferation of tumor cells; (2) suppress activation of T-cells by antigen or IL-2 stimulation; (3) suppress activation of monocyte/macrophage cells by endotoxin, TNF, IL-1 or GM-CSF stimulation; (4) suppress antibody production of B-cells in response to an antigen, IL-4 or CD40 ligand; (5) inhibit proliferation of smooth muscle cells in response to growth factors capable of stimulating said proliferation; (6) lower systemic vascular resistance conferred by endothelial cells by reducing release of hypertension-inducing substances; (7) lower systemic vascular resistance induced by endothelial cells by enhancing release of anti-hypertensive substances; (8) lower expression of adhesion molecules induced by enhancers thereof; (9) suppress activation of T-cells and macrophages by HIV, thus inhibiting viral replication; (10) inhibit proliferation of kidney mesangial cells in response to stimulation by IL-1 and/or MIP-1.alpha. and/or PDGF and/or FGF; (11) enhance resistance of kidney glomerular or tubular cells to cyclosporin A or amphotericin B; (12) prevent release of MIP-1.alpha. by IL-1, TNF, or endotoxin stimulated monocytes and macrophages; (13) prevent release of platelet activating factor by IL-1, TNF, or endotoxin treated megakaryocytes, fibroblastic cells, and macrophages; (14) prevent down-regulation of receptors for cytokines in TNF-treated hematopoietic progenitor cells; (15) suppress production of metalloproteases in IL-1- or TNF-stimulated glomerular epithelial or synovial cells; (16) enhance resistance of gastrointestinal or pulmonary epithelial cells to cytotoxic drugs or radiation; (17) enhance the antitumor effect of a non-alkylating antitumor agent; (18) to inhibit production of osteoclast activating factor in response to IL-1; (19) inhibit degranulation in response to IgE; (20) enhance release of adrenergic neural transmitters, dopamine, norepinephrine, or epinephrine, or the neurotransmitter, acetylcholine; (21) modulate post-synaptic "slow current" effects of adrenergic neurotransmitters, such as, dopamine, epinephrine, or norepinephrine, or the neurotransmitter acetylcholine; (22) suppress signaling by neurotransmitters including acetyl choline, leuenkephalin and serotonin; or (23) increase seizure threshold.

The compounds of the invention can inhibit certain VEGF, FGF, EGF and PDGF effects in vivo, such as inhibition of angiogenesis or restenosis. For example, Ferns et al. ("Inhibition of Neointimal Smooth Muscle Accumulation After Angioplasty by an Antibody to PDGF," Science, Vol. 253, pages 1129-1132, 1991) have shown that neointimal smooth muscle chemotaxis and angioplasty are inhibited in rats using a neutralizing antibody to PDGF. Also, Jawien et al. ("Platelet-derived Growth Factor Promotes Smooth Muscle Migration and Intimal Thickening in a Rat Model of Balloon Angioplasty," J. Clin Invest, Vol. 89, pages 507-511, 1992) have shown that PDGF promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. Inhibition of the PDGF-mediated effects following balloon angioplasty by the inventive compounds is the pharmacological rationale for using the inventive compounds as therapeutic agents to prevent restenosis. The inventive compounds also inhibit atherogenesis because increased levels of PDGF expressed by macrophages are associated with all phases of atherogenesis (Ross et al., "Localization of PDGF-B Protein in Macrophages in All Phases of Atherogenesis," Science, Vol. 248, pages 1009-1012, 1990). Further, many human tumors express elevated levels of either PDGF, FGF, receptors for FGF or PDGF, or mutated cellular oncogenes highly homologous to these growth factors or their receptors. For example, such tumor cell lines include sarcoma cell lines (Leveen et al., "Expression of Messenger RNAs for Platelet-Derived Growth Factor and its Receptors in Human Sarcoma Cell Lines," Int. J. Cancer, Vol. 46, pages 1066-1070, 1990), metastatic melanoma cells (Yamanishi et al., "Differences in Basic Fibroblast Growth Factor RNA and Protein Levels in Human Primary Melanocytes and Metastatic Melanoma Cells," Cancer Research, Vol. 52, pages 5024-5029, 1992), and glial tumors (Fleming et al., "Amplification and/or Overexpression of Platelet-derived Growth Factor Receptors and Epidermal Growth Factor Receptor in Human Glial Tumors," Cancer Research, Vol. 52, pages 4550-4553, 1992).

The inventive compounds are also useful to raise the seizure threshold, to stabilize synapses against neurotoxins such as strychnine, to potentiate the effect of anti-Parkinson drugs such as L-dopa, to potentiate the effects of soporific compounds, to relieve motion disorders resulting from administration of tranquilizers, and to diminish or prevent neuron overfiring associated with progressive neural death following cerebral vascular events such as stroke. In addition, the compounds of the invention are useful in the treatment of norepinephrine-deficient depression and depressions associated with the release of endogenous glucocorticoids, to prevent toxicity to the central nervous system of dexamethasone or methylprednisolone, and to treat chronic pain without addiction to the drug. Further, the compounds of the invention are useful in the treatment of children with learning and attention deficits and may generally improve memory in subjects with organic deficits, including Alzheimer's patients.

Compounds of the Invention

The inventive compounds are useful therapeutic agents, inhibiting proinflammatory and neoplastic cellular signaling mechanisms and have the formula:

CORE MOIETY--(R).sub.j

including resolved enantiomers and/or diastereomers, hydrates, salts, solvates and mixtures thereof. In this formula, j is an integer from one to three, the core moiety is non-cyclic or cyclic and R may be an amine, hydrogen, halogen (preferably bromine, chlorine, fluorine and iodine), hydroxyl, substituted or unsubstituted C.sub.(1-10) alkyl, C.sub.(2-10) alkenyl, cyclic or heterocyclic group or formula I: ##STR3##

Preferred R substituents other than formula I include, but are not limited to, 2-bromopropyl, 4-chloropentyl, cyclohexyl, cyclopentyl, 3-dimethylaminobutyl, ethyl, hexyl, 2-hydroxyethyl, 5-hydroxyhexyl, 3-hydroxy-n-butyl, 3-hydroxypropyl, isobutyl, isopropyl, 2-methoxyethyl, 4-methoxy-n-butyl, methyl, n-butyl, n-propyl, phenyl, t-butyl and the like. Particularly preferred R having a structure other than formula I are ethyl, methyl, or hydrogen.

The inventive compounds have at least one R of formula I. In formula I, n is an integer from four to twenty; each R.sub.1 or R.sub.2 is independently hydrogen, substituted or unsubstituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl group, or cyclic or heterocyclic group, the alkyl or alkenyl being preferably substituted by an aryl, halogen or ketone group. Preferably, n is an integer from four to fourteen or and more preferably n is an integer from six to ten. Optionally, (CH.sub.2).sub.n may 1) be substituted by a substituted or unsubstituted C.sub.(1-10) alkyl or C.sub.(2-10) alkenyl group; or 2) have one or two unsaturated bonds (preferably in a cis configuration). In most preferred compounds of the invention, R.sub.1 and R.sub.2 are both hydrogen or methyl or one of R.sub.1 or R.sub.2 is hydrogen and the other of R.sub.1 or R.sub.2 is an unsubstituted C.sub.(1-20) alkyl or a C.sub.(1-20) alkyl substituted by an unsubstituted heterocycle.

Although other possible substituents are within the scope of the inventive compounds, when R.sub.1 or R.sub.2 is a substituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl or cyclic or heterocyclic group, representative substituents may be selected from among amide, primary, secondary and tertiary amine, C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl (including, e.g., branched and unbranched alkyl or alkenyl groups), C.sub.(1-8) alkoxyl, C.sub.(1-8) hydroxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, C.sub.(1-8) haloalkyl, (including, e.g., mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanate, isothiocyanate, phosphate, phosphonate, sulfonate, sulfone, sulfoxide, mercaptocarbonyl, and mercaptocarbonato group thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea Moreover, when R.sub.1 or R.sub.2 is a substituted C.sub.(1-20) alkyl, C.sub.(1-20) alkoxyl, C.sub.(2-20) alkenyl, representative substituents also include aryl, halogen and oxo.

When (CH.sub.2).sub.n is branched by a substituted C.sub.(1-10) alkyl or C.sub.(2-10) alkenyl group, corresponding substituents may also be selected from the foregoing list.

Representative R.sub.1 or R.sub.2 cyclic or heterocyclic groups include, but are not limited to: anthracene, bicyclo[4.4.0]decane, bicyclo[2.2.1]heptane, bicyclo[3.2.0]heptane, bicyclo[4.1.0]heptane, bicyclo[2.2.1] hexane, bicyclo[4.3.0]nonane, bicyclo[2.2.2]octane, biphenyl, cyclopentadiene, cyclopentane, cyclobutane, cyclobutene, cycloheptane, cyclohexane, cyclooctane and cyclopropane, 1,2-diphenylethane, fluorene, indene, phenyl, quinone, terphenyl, napthalene, phenanthrene, terphenyl, toluene, xylene, azetidine, benzofuran, benzothiophene, carbazole, furan, glutarimide, indole, isoquinoline, lactam, lactone, oxazole, oxetane, oxirane, phthalimide, piperidine, pyrrolidine, pyran, pyridine, pyrrole, quinoline, tetrahydrofuran, tetrahydropyran, tetrahydrothiophene, thiophene, thymine, derivatives thereof and the like. Due primarily to

availability and ease of synthesis, more preferred cyclic groups include, but are not limited to, less complex ring systems, such as, for example, cyclopentane and cyclohexane, cyclopentadiene, phenyl, indene, toluene, xylene, furan, indole, thymine and xanthine.

The inventive compounds are active therapeutic agents by virtue of an ability to prevent a second messenger from effecting an undesirable cell response. The core moiety serves as an orienting or plasma membrane-anchoring moiety. The orienting moiety may spacially orient the (R) structural component(s) of the inventive compounds, having the appropriately-substituted amine functional group, to an active site of an enzyme involved in phospholipid-based second messenger cellular signaling. Therefore, a large number of core moieties are active by virtue of their ability to orient a compound in a cellular plasma membrane.

A non-cyclic core moiety may include, but is not limited to, for example, acetamide, amide, amine, amino acid (one or two), carboxide, ester, terminal halogen or hydrogen atom, hydroxide, glutaric acid, glycine derivative, ketone, phosphate, phosphonate, sulfate, sulfonate, sulfone, sulfoxide, simple ionic functional group, thiol, thiolester or the like. Exemplary core moiety amino acids may include, but are not limited to, one or more of the following: alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. The non-cyclic core moiety may preferably be an amide, carboxyl ester, carboxide, hydrogen, hydroxide or a dipeptide comprising two amino acids selected from the foregoing exemplary list. A non-cyclic, halogen-core moiety may be, for example, bromine, chlorine, fluorine or iodine.

A cyclic core may be at least one five- to seven-member, non-heterocyclic (i.e., carbocyclic) ring or a heterocycle. The at least one five- to seven-membered cyclic core may preferably have from one to three, five- to six-membered ring structures in a predominantly planar configuration. An exemplary, non-heterocyclic ring core moiety may be selected from the group consisting of substituted or unsubstituted benzene; biphenyl; cyclohexane; cyclohexanedione; cyclopentanedione; napthlalene; phenol; quinone; salicylic acid; stilbene and tricyclododecane.

Although other heterocyclic cores are within the scope of the invention, the following representative cores are preferred: substituted or unsubstituted barbituric acid; benzamide; lactam; glutarimide; homophthalimide; hydrophthalimide; imidazole; imidazole amide; indomethacin; isocarbostyril; lumazine; N-alkylheterocyclic; N-heterocyclic; pteridine; pthalimide; piperidine; pyridine; pyrimidine; pyrrole amide; quaternized N-heterocyclic; quinolizinedione; quinazolinone; quinoline; resorcinol; succinimide; theobromine; thymine; triazine; uric acid; uracil; vitamins A, E or K; or xanthine.

Representative substituents for the non-heterocyclic or heterocyclic core moieties include, but are not limited to, for example, amide, primary, secondary and tertiary amine, C.sub.(2-8) alkenyl, C.sub.(1-8) alkyl (including, e.g., branched and unbranched alkyl or alkenyl groups), C.sub.(1-8) alkoxyalkyl, azide, carbonate, carbonyl, carboxylic acid, cyanide, C.sub.(1-8) haloalkyl (including, e.g., mono-, di- and tri-haloalkyl substituents, such as trihalomethyl), isocyanate, isothiocyanate, phosphate, phosphonate, primary, secondary or tertiary alcohol (including, e.g., any one of various diols, methanol, butanol, 1-cyclopentanol, ethanol, 2-ethyl-3-methyl-1-propanol, pentanol, propanol, and methylcyclohexanol), sulfonate, sulfone, sulfoxide, thioamide, thiocarbonate, thioester, thiolester, thiol, thiourea and urea.

Preferred non-heterocyclic ring cores include, but are not limited to, substituted or unsubstituted 1,3-cyclohexanedione, 1,3-cyclopentanedione; 1,3-dihydroxynaphthalene; or orthophenol.

Preferred heterocyclic cores include, but are not limited to, substituted or unsubstituted 3,7-dimethylxanthine, glutarimide, methylthymine, methyluracil, 3-methylxanthine, thymine, uracil and xanthine, most preferably methyl-substituted xanthine. Exemplary preferred cores include, but are not limited to: C.sub.(1-6) alkyl-substituted thymine; C.sub.(1-6) alkyl-substituted uracil; 1,3-dihydroxynapthalene; 3,3-dimethylglutarimide; dihydrothymine; 2,4-dioxohexahydro-1,3,5-tetrazine; hexahydrophthalimide; homophthalimide; 2-hydroxypyridine; .beta.-ionone as vitamin A methylbarbituric acid; 2,6,6-methyl-1-cyclohexene-1-acetaldehyde as vitamin A; methyldihydroxypyrazolopyrimidine, specifically, 1,3-dimethyldihydroxypyrazolo [4,3-d]pyrimidine; 1-methyl-5,6-dihydrouracil; 1,7-dimethylxanthine, 3,7-dimethylxanthine; 7-methylhypoxanthine; 1-methyllumazine; 3-methyl-7-methylpivaloylxanthine; methylpyrrolopyrimidine; 1-methylpyrrolo [2,3-d]pyrimidine; 1-methyl-2,4(1H,3H)-quinolizinedione (1-methylbenzoyleneurea); methylthymine; 1-methyluracil; 3-methylxanthine; orotic acid; prostacyclin; 1-pyrrole amides; 2-pyrrole amides; 3-pyrrole amides; quinazolin-4(3H)-one; 1,2,3,4-tetrahydroisoquinoline; tetrahydrophthalimide; sulindac; uracil fused to naphthalene; 5- and/or 6-position substituted uracils (such as, for example, 5-bromouracil); tetralone to vitamin K; and 8-substituted xanthines (having substituents such as N or S).

Preferably, R is bonded to a nitrogen of the core moiety, if present, most preferably to the nitrogen of a glutarimide, methylthymine, thymine, uracil or xanthine core. In representative, preferred compounds, R of formula I may be bonded to an N.sub.1 nitrogen of glutarimide; N.sub.1 nitrogen of xanthine (and N.sub.3 and N.sub.7 xanthine nitrogens may be independently substituted by a member selected from the group consisting of hydrogen, C.sub.(1-6) alkyl, fluoro, chloro and amino); N.sub.3 nitrogen of methylthymine; or N.sub.1 nitrogen of uracil. Alternatively, R having formula I may be bonded to N.sub.1 and N.sub.3 xanthine nitrogens and the N.sub.7 xanthine nitrogen is substituted by a member selected from the group consisting of hydrogen, methyl, fluoro, chloro and amino.

Particularly preferred compounds of the invention are exemplified herein.

Synthesis of the Inventive Compounds

The invention is also directed to a method for preparing compounds according to the invention. The method is discussed in general below and in specific detail in the examples.

In the inventive method, a compound containing a desired core (intended as the "core moiety") undergoes a reaction to produce an anion, which is then subsequently reacted with a substituted ester, displacing a target functional group of the ester. A predetermined amount of the core-containing compound is reacted with a suitable base and the substituted ester in a solvent to form and ester product. The substituted ester has at least one functional group which may be substituted by the desired core-containing compound in the displacement reaction.

Preferred bases include, but are not limited to, sodium hydride, sodium amide, sodium alkoxide, lithium hydride, potassium hydride, lithium amide and potassium amide. An especially preferred base is sodium hydride. Preferred solvents may be dimethylsulfoxide, dimethylformamide, or an alcohol, such as, for example, methanol, ethanol or isopropanol. Any substituted ester comprising a chain structure of the final inventive compounds may be used. Preferred substituted esters include, but are not limited to halo-substituted esters.

The ester product, having a composite structure of the core-containing compound and substituted ester, may subsequently be converted by reacting it with an ester-hydrolyzing agent to obtain an intermediate carboxylic acid. Exemplary ester-hydrolyzing agents include, but are not limited to, potassium hydroxide or sodium hydroxide in water. The intermediate carboxylic acid is then reacted in a halogenation reaction with a halogenating agent to obtain a compound having a carboxylic acid halide functional group. Exemplary halogenating agents include, but are not limited to, thionyl chloride, phosphorus trichloride, phosphorus pentachloride, phosphorus oxychloride, thionyl bromide and the like. To obtain a substituted amide functional group, the compound having a carboxylic acid halide functional group is reacted with a substituted amine.

By then reacting the substituted amide with a suitable reducing agent, the substituted amide is reduced to the corresponding inventive amine-substituted compound. Exemplary reducing agents include, but are not limited to, borane-tetrahydrofuran complex and diisobutylaluminumhydride.

Alternatively, a compound containing a desired core (intended as a "core moiety") undergoes a reaction to produce a halide product. A core-containing compound is reacted in a solvent with a suitable base and a substituted halide, the substituted halide having at least one other functional group which may be substituted in a displacement reaction by the desired core-containing compound.

In this reaction, preferred bases include, but are not limited to, sodium hydride, sodium amide, sodium alkoxide, lithium hydride, potassium hydride, lithium amide, sodium amide and potassium amide. An especially preferred base is sodium hydride. Preferred solvents may be dimethylsulfoxide, dimethylformamide, or an alcohol, such as, methanol, ethanol or isopropanol. Any substituted halide comprising a chain structure of the inventive compounds may be used in the preliminary reaction according to the invention. Preferred substituted halides may be halo-substituted halides (or dihalides).

The halide product, having a composite structure of the core-containing compound and substituted halide, may be converted to a corresponding compound having an azido group. The halide product is reacted with a salt of hydrazoic acid to obtain an azide. Hydrazoic acid salts may be selected from potassium azide, sodium azide, or lithium azide. Reducing the substituted azide using a suitable reducing agent results in an inventive compound. Reducing agents include, but are not limited to, hydrogen with palladium on carbon, hydrogen with Raney nickel, or hydrogen with platinum oxide.

Additionally, a substituted aldehyde or ketone, having a composite structure of the core-containing compound, and either a substituted aldehyde or a substituted ketone may be converted to an inventive compound in a reductive amination reaction using a substituted amine and suitable reducing agent. Exemplary reducing agents include, but are not limited to, sodium cyanoborohydride and sodium borohydride. Schematic representations of the inventive methods discussed above are illustrated in schematics A, B and C below: ##STR4##

The compounds of the invention may be provided as enantiomeric or diastereomeric mixtures or in resolved or partially resolved forms. Standard procedures are used for resolving optical isomers. Different enantiomeric variants (e.g., stereoisomers and chiral forms) of the inventive compound may have different drug activities, based upon their differential ability to inhibit PAPH and LPAAT. An optical isomer, substantially free of the corresponding enantiomer and/or diastereomers, is at least about 85% of a relevant optical isomer, preferably at least about 95% relevant optical isomer and especially at least about 99% or higher relevant optical isomer. Most preferably an amount of other optical forms is undetectable.

The invention provides a pharmaceutical composition comprising an inventive compound and a pharmaceutically acceptable excipient. The pharmaceutical composition may be formulated for oral, parenteral or topical administration to a patient.

The invention further provides a pharmaceutical composition comprising an inventive compound and a pharmaceutically acceptable excipient, the pharmaceutical composition being formulated for oral, parenteral or topical administration to a patient. A pharmaceutical composition may alternatively comprise one or a plurality of inventive compounds and a pharmaceutically acceptable carrier or excipient. Treatment of individuals with the inventive compound or pharmaceutical composition may include, but is not limited to, contacting with the inventive compound in vitro culture, in an extracorporeal treatment, or by administering (oral, parenteral or topical) the inventive compound or pharmaceutical composition to a subject whose cells are to be treated.

Exemplary, preferred compounds of the invention include, but are not limited to, both R and S enantiomers and racemic mixtures of the following compounds: ##STR5##

Pharmaceutical Formulations

A suitable formulation will depend on the nature of the disorder to be treated, the nature of the medicament chosen, and the judgment of the attending physician. In general, the inventive compounds are formulated either for injection or oral administration, although other modes of administration such as transmucosal or transdermal routes may be employed. Suitable formulations for these compounds can be found, for example, in Remington's Pharmaceutical Sciences (latest edition), Mack Publishing Company, Easton, Pa.

The inventive compounds and their pharmaceutically acceptable salts can be employed in a wide variety of pharmaceutical forms. The preparation of a pharmaceutically acceptable salt will be determined by the chemical nature of the compound itself, and can be prepared by conventional techniques readily available. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1 gram, wherein the amount of inventive compound per dose will vary from about 25 mg to about 1 gram for an adult. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension. Where the inventive composition is in the form of a capsule, any routine encapsulation is suitable, for example, using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule, any pharmaceutical carrier routinely used for preparing dispersions of suspensions may be considered, for example, aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell. A syrup formulation will generally consist of a suspension or solution of the compound or salt thereof in a liquid carrier (e.g., ethanol, polyethylene glycol, coconut oil, glycerine or water) with a flavor or coloring agent.

The amount of inventive compound required for therapeutic effect on topical administration will, of course, vary with the compound chosen, the nature and severity of the disease and the discretion of the treatment provider. Parenteral includes intravenous, intramuscular, subcutaneous, intranasal, intrarectal, intravaginal or intraperitoneal administration. Appropriate dosage forms for such administration may be prepared by conventional techniques. A typical parenteral composition consists of a solution or suspension of the inventive compound or a salt thereof in a sterile or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil. The daily dosage

for treatment of sepsis or another severe inflammatory condition by parenteral administration from about 0.001 mg/kg to about 40 mg/kg, preferably from about 0.01 mg/kg to about 20 mg/kg of an inventive compound or a pharmaceutically acceptable salt thereof, calculated as the free base.

The inventive compounds may be administered orally. The daily dosage regimen for oral administration is suitably from about 0.1 mg/kg to about 1000 mg/kg per day. For administration the dosage is suitably form about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof calculated as the free base. The active ingredient may be administered from 1 to 6 times a day, sufficient to exhibit activity.

The inventive compounds may be administered by inhalation (e.g., intranasal or oral). Appropriate dosage forms include, but is not limited to, an aerosol or a metered dose inhaler, as prepared by conventional techniques. The daily dosage is suitably form about 0.001 mg/kg to about 40 mg/kg of the inventive compound or a pharmaceutically acceptable salt thereof calculated as the free base. Typical compounds for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant.

While dosage values will vary, therapeutic efficacy is achieved when the compounds of the invention are administered to a human subject requiring such treatment as an effective oral, parenteral, or intravenous dose of about 50 mg to about 5000 mg per day, depending upon the weight of the patient. A particularly preferred regimen for use in treating leukemia is 4-50 mg/kg body weight. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual's need and to the professional judgment of the person administering or supervising the administration of the inventive compounds.

The following examples, which should not be regarded as limiting in any way, further illustrate the invention.

EXAMPLE 1

This example is a method of synthesis for inventive compound no. 3506 (see above for chemical name and structure). A mixture of theobromine (1.0 g, 5.5 mmol, available from Sigma) and 50% sodium hydride in oil (264 mg, 5.5 mmol) in dimethylsulfoxide (20 ml) was stirred for 50 minutes and then 6-bromo-1-hexanol (1.0 g, 5.5 mmol, available from Aldrich) was added. After stirring for 18 hours, the solution was treated with water (50 ml) and then extracted with two 25 ml aliquots of hexanes. The aqueous phase was extracted with three 35 ml aliquots of 25% ethanol-dichloromethane. The combined ethanol-dichloromethane extracts were dried over magnesium sulfate. The solvents were then evaporated under vacuum and remaining dimethylsulfoxide was removed by distillation under full pump vacuum, leaving 1.4 g of a white powder, 1-(6-hydroxyhexyl)-3,7-dimethylxanthine (5.0 mmol, 91% yield).

Dimethyl sulfoxide (156 .mu.l, 172 mg, 2.2 mmol) was slowly added to a solution of oxalyl chloride (103 .mu.l, 150 mg, 1.2 mmol) in dichloromethane at -78.degree. C. A solution of 1-(6-hydroxyhexyl)-3,7-dimethylxanthine (300 mg, 1.1 mmol), prepared in the previous step, in dichloromethane (5 ml) was added to this solution and the resulting reaction mixture stirred for 15 minutes. The cold bath was removed after addition of triethylamine (765 .mu.l, 555 mg, 5.5 mmol). The reaction was added at ambient temperature to 20 ml water and extracted with three 50 ml aliquots of methylene chloride. The combined organic layers were washed with 1% aqueous hydrogen chloride (20 ml), saturated aqueous sodium bicarbonate (20 ml), and saturated aqueous salt solution (20 ml), and then dried over sodium sulfate. Evaporating the solvents and recrystallizing a residue in chloroform/petroleum ether produced 267 mg of 1-(6-oxohexyl)-3,7-dimethylxanthine (87% yield).

Then, sodium cyanoborohydride (63 mg, 1.0 mmol) was added to a mixture of 1-(6-oxohexyl)-3,7-dimethylxanthine (150 mg, 0.5 mmol), prepared above, undecylamine (0.43 ml, 2.5 mmol), 38% aqueous hydrochloric acid solution (0.2 ml, 2.5 mmol), methanol (5 ml), and THF (5 ml) and the resulting solution stirred for 48 hours. Saturated aqueous ammonium chloride solution (20 ml) was added to the stirring solution. Following an additional 20 minutes of stirring, 30 ml of 30% aqueous ammonium hydroxide solution were added. The mixture was extracted with three 35 ml aliquots of 25% methanol-dichloromethane. The combined extracts were dried over sodium sulfate and the solvents were evaporated under vacuum, producing 190 mg of compound no. 3506 (86% yield).

EXAMPLE 2

This example is a method of synthesis for inventive compound no. 3556. A solution of 11-bromoundecanoic

acid (available from Aldrich, 5.70 g, 22 mmol) and p-toluenesulfonic acid (0.1 g) in absolute ethanol (100 ml) was refluxed for 3 hours. Saturated aqueous sodium bicarbonate solution (40 ml) was added and the reaction mixture was extracted with dichloromethane (3.times.70 ml). The combined extracts were washed with water (50 ml) and saturated aqueous salt solution (50 ml), and then the solvent was evaporated to a colorless oil. Ethyl 11-bromoundecanoate (5.92 g, 94% yield) was collected during distillation (2 mm) at 135.degree. C. A solution of the bromoester (5.92 g, 20 mmol) and 1-sodiotheobromine (4.08 g, 20 mmol) in dimethylsulfoxide (80 ml) was stirred for 18 hours at ambient temperature. The mixture was added to water (100 ml) and dichloromethane (100 ml). The aqueous layer was extracted with dichloromethane (2.times.80 ml). The combined organic layers were washed with water (80 ml) and saturated aqueous salt solution (80 ml), dried over magnesium sulfate, and evaporated under vacuum to a white solid. The residue was recrystallized in dichloromethane/ether/hexane, yielding 4.95 g of 1-(ethyl 11-yl-undecanoate)-3,7-dimethylxanthine (62% yield).

A solution of potassium hydroxide (0.50 g, 9.0 mmol) in water (1 ml) was added to a stirring suspension of 1-(ethyl 11-yl-undecanoate)-3,7-dimethylxanthine (2.52 g, 6.4 mmol) in methanol (15 ml). The mixture was warmed until it became homogeneous, and the stirring was continued overnight at ambient temperature. Water (10 ml) was added to the reaction mixture followed by a 5% solution of sulfuric acid (10 ml). The precipitate was filtered off and washed with ether, then dried under vacuum to obtain 2.12 g of 1-(11-yl-undecanoic acid)-3,7-dimethylxanthine (91% yield).

A solution of 1-(11-yl-undecanoic acid)-3,7-dimethylxanthine (1.62 g, 4.5 mmol) and thionyl chloride (0.5 ml, 6.7 mmol) in toluene (5 ml) was heated at 80.degree. C. for 1 hour and then cooled. The solvent was evaporated under a nitrogen stream. The resulting acid chloride was taken up in dichloromethane (20 ml), and 1-octylamine (2 ml, 11 mmol) was added by syringe to the stirring solution. After 2 hours, water (50 ml) was added and the mixture was extracted with three 50 ml aliquots of dichloromethane. The combined organic extracts were washed with 5% hydrochloric acid (100 ml) and saturated aqueous salt solution (60 ml) and then dried over sodium sulfate. The solvent was evaporated under vacuum, leaving a residue was purified by column chromatography using basic activity II alumina and a dichloromethane/10% methanol eluant producing 1.47 g of white solid, 1-(N-octyl 11-yl-undecanamide)-3,7-dimethylxanthine (69% yield).

A 1 M solution of borane-tetrahydrofuran (6 ml, 6 mmol) was added dropwise to a stirring solution of 1-(N-octyl-11-yl-undecanoamide)-3,7-dimethylxanthine (0.85 g, 1.8 mmol), prepared above, in tetrahydrofuran (10 ml) under argon. After 3 hours of stirring at reflux, the reaction mixture was cooled to ambient temperature and 6 M aqueous hydrogen chloride (4 ml) was added dropwise, resulting in a foaming reaction mixture. After bubbling subsided, most of the solvent was removed under a stream of argon. Water (20 ml) was added, and saturated aqueous sodium bicarbonate solution was added dropwise until the aqueous mixture was at a Ph of approximately 8, using Ph paper to test. The mixture was extracted with three 40 ml aliquots of dichloromethane. The combined organic layers were evaporated under vacuum, leaving a white solid residue. This residue was purified by column chromatography using neutral activity II alumina and a dichloromethane/3% methanol eluant, producing 0.66 g of a white solid, 1-(11-octylaminoundecyl)-3,7-dimethylxanthine (80% yield).

EXAMPLE 3

This example is a method of synthesis for inventive compound no. 3563 (see above for chemical name and number). 1-(11-Yl-undecanoic acid)-3,7-dimethylxanthine was prepared as described in the synthetic protocol of example 2. Thionyl chloride (0.6 ml, 8.2 mmol) was added to a slurry of 1-(11-yl-undecanoic acid)-3,7-dimethylxanthine (2.12 g, 5.8 mmol) in toluene (5 ml) under argon. The stirring mixture was warmed to 80.degree. C., and became homogeneous. After 1 hour, the solvent was evaporated under a stream of argon, to give the acid chloride as an off-white solid residue. This compound was used in the next step without further purification. The acid chloride was taken up in dichloromethane (20 ml) and added dropwise to a stirring solution of dodecylamine (4.3 g, 23 mmol) in dichloromethane (20 ml). After 2 hours of stirring at ambient temperature, 3% aqueous hydrogen chloride solution (100 ml) and water (50 ml) was added to the resulting slurry. The mixture was extracted with dichloromethane-5% methanol (3.times.70 ml). The combined organic layers were washed with saturated aqueous salt solution (70 ml) and dried over magnesium sulfate. The solvents were evaporated under vacuum to give a white solid residue. The solid was purified by column chromatography (silica/dichloromethane-5% methanol), yielding 2.08 g of a white solid, 1-(N-dodecyl-11-yl-undecanoamide)-3,7-dimethylxanthine (68% yield).

A 1 M solution of borane-tetrahydrofuran (2 ml, 2 mmol) was added dropwise to a stirring solution of 1-(N-dodecyl-11-yl-undecanoamide)-3,7-dimethylxanthine (0.30 g 0.6 mmol), prepared above, in tetrahydrofuran (5 ml) under argon. After 2 hours of stirring at reflux, the reaction mixture was cooled to ambient

temperature and 6 M aqueous hydrogen chloride (0.6 ml) was added dropwise. After bubbling subsided, the solvent was mostly removed under a stream of argon. Water (10 ml) and dichloromethane (20 ml) was added, and saturated aqueous sodium hydroxide solution was dripped in until the aqueous layer showed a Ph of approximately 10 using pH paper. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2.times.60 ml). The combined organic layers were evaporated under vacuum to a white solid. This residue was purified by chromatography (neutral activity II alumina, dichloromethane-3% methanol), resulting in 240 mg of a white solid, inventive compound no. 3563 (77% yield).

EXAMPLE 4

This example is a method of synthesis for inventive compound no. 4500. To a slurry of 1-(11-yl-undecanoic acid)-3,7-dimethylxanthine (2.0 g, 5.5 mmol), prepared as an intermediate in example 2, in toluene (10 ml) under argon was added thionyl chloride (0.6 ml, 8.2 mmol). The stirring mixture was warmed to 80.degree. C., and became homogeneous. After 1 hour, the excess thionyl chloride was removed under a stream of argon and the solvent was removed under reduced pressure, to give the acid chloride as an off-white solid. This compound was used in the next step without further purification. The acid chloride was taken up in dichloromethane (20 ml) and added dropwise to a stirring solution of 1-hexylamine (2.1 ml, 16 mmol) in dichloromethane (20 ml). After 2 hours of stirring at ambient temperature, the reaction was poured into 3% aqueous hydrogen chloride solution (100 ml) followed by saturated aqueous salt solution (40 ml). The mixture was extracted with dichloromethane (3.times.50 ml). The combined organic layers were washed with saturated aqueous salt solution (50 ml) and dried over magnesium sulfate. The solvents were evaporated under vacuum to give a white solid residue. The solid obtained was purified by column chromatography (silica/dichloromethane-5% methanol), resulting in 1.52 g of a white solid, 1-(N-hexyl-11-yl-undecanamide)-3,7-dimethylxanthine (62% yield).

To a stirring solution of 1-(N-hexyl-11-yl-undecanamide)-3,7-dimethylxanthine (1.0 g, 2.3 mmol), prepared above, in tetrahydrofuran (15 ml), cooled to 0.degree. C. under argon was slowly added borane-tetrahydrofuran complex (6.7 ml, 6.7 mmol). The cold bath was removed and the reaction was heated to 70.degree. C. After 3 hours the reaction was cooled to ambient temperature and 6 molar hydrochloric acid (6 ml) was slowly added. The tetrahydrofuran was removed by distillation at atmospheric pressure. To the remaining cooled aqueous solution was added dichloromethane (30 ml), a saturated solution of sodium hydroxide (10 ml) and water (20 ml). The basic aqueous solution was extracted with dichloromethane (3.times.25 ml). The organic extracts were dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure gave a crude white residue. Column chromatography on neutral activity II alumina (ethyl acetate/10% methanol) yielded 0.71 g of 1-(11-hexylaminoundecyl)-3,7-dimethylxanthine (73% yield) as a white solid.

Formic acid (0.23 ml, 6.1 mmol) was added to 1-(11-hexylaminoundecyl)-3,7-dimethylxanthine (300 mg, 0.7 mmol), prepared above. A 37% aqueous solution of formaldehyde (0.41 ml, 5.4 mmol) was added and the reaction solution was stirred at 90.degree. C. for 24 hours. After cooling to ambient temperature, a saturated solution of sodium carbonate (15 ml) was added. The basic aqueous solution was extracted with dichloromethane (3.times.15 ml). The organic extracts were collected and dried over anhydrous magnesium sulfate. Solvent was removed under reduced pressure to give a crude yellow oil. Column chromatography on neutral activity alumina with ethyl acetate/10% methanol/10% trimethylamine as eluant produced 180 mg of a colorless oil, which solidified upon standing, inventive compound no. 4500 (180 mg, 54% yield).

EXAMPLE 5

This example shows the effects of inventive compound no. 3506 on PDGF-induced proliferation in human stromal cells. Procedurally, human stromal cells were starved in serum-free media for 24 hours and then stimulated with 50 ng/ml PDGF. Compound no. 3506 was added at various concentrations one hour prior to PDGF stimulation and pulsed for 24 hours. Cells were harvested and cell proliferation measured having background counts (i.e., starved cells) at about 10% of control levels. FIG. 1 illustrates the inventive compound's inhibition of PDGF-induced proliferation at various concentrations (.mu.M).

EXAMPLE 6

This example illustrates inhibitive effects of the inventive compounds on Balb/3T3 cell proliferation in response to platelet derived growth factor (PDGF) stimulation.

Disregulated PDGF-proliferative response has been linked to a variety of diseases, including, e.g., restenosis, atherosclerosis, fibrosis, and tumor cell angiogenesis. Balb/3T3 cells respond vigorously to PDGF stimulation,

and are useful in vitro models for further study of PDGF-induced proliferation. In an assay useful in determining whether a compound would be useful in treating diseases characterized by this or similar disregulated proliferative responses, research indicates that the inventive compounds inhibit PDGF-induced proliferation of Balb/3T3 cells.

Balb/3T3 cells were plated in low serum-containing medium for 24 hours prior to stimulation with various concentrations of inventive compounds nos. 3556 and 4500. PDGF was added at varying concentrations along with tritiated thymidine. The cells were allowed to incubate for one day, following addition of PDGF and thymidine. Twenty-four hours later, the cells were harvested and counted by liquid scintillation counting. FIGS. 2 and 4 report data for inventive compounds nos. 3556 and 4500, respectively, obtained in this proliferation assay. The results shown in each respective figure illustrate that inventive compounds nos. 3556 and 4500 inhibit proliferation of Balb/3T3 cells stimulated by PDGF at concentrations less than 30 .mu.M, indicating that the inventive compounds are candidates for treating or preventing restenosis, atherosclerosis, fibrosis, tumor cell angiogenesis and other similar diseases.

In conjunction with the Balb/3T3 proliferation assay, a related viability assay was conducted to assess the cytotoxicity of compounds which inhibit proliferation in this system. The assay protocol was identical to that performed above except that tritiated thymidine was not added after the 24 hour incubation with PDGF. In this cytotoxicity assay, after incubating the cells, a 10 .mu.M solution of BCECF was added and the cells were incubated for 30 minutes at 37.degree. C. Following this incubation, BCECF was replaced with PBS and the plate read for fluorescence in a Millipore cytofluorometer. Data obtained was plotted as a percent of control versus concentration of inventive compound tested. FIGS. 3 and 5 represent the results of this viability assay, for compounds nos. 3556 and 4500, respectively. The compounds tested (compounds nos. 3556 and 4500) were not cytotoxic to any cells (as compared with a control value of 100%) at concentrations shown in FIGS. 1, 2 and 4, the concentrations at which the respective compounds inhibit proliferation.

EXAMPLE 7

This example shows an inhibitive effect of inventive compounds nos. 3506, 3556, 3563, 3576, 3581, 3582 and 3584 on thymocyte proliferation and activation, co-stimulated with Concanavalin A (Con A) and interleukin-2 (IL-2), at various concentrations of the compounds (IC.sub.50). Con A and IL-2 together stimulate T cell proliferation and differentiation.

Thymuses, obtained from normal, female Balb/C mice, were dissociated and plated into 96-well plates at a density of 2.times.10.sup.5 cells/well. Con A (0.25 mg/ml) and IL-2 (15 U/ml) were added to the wells. The cells were incubated for 4 days at 37.degree. C. On day 4, the cells were pulsed with tritiated thymidine and incubated for an additional 4 hours. Incorporated tritiated thymidine of harvested cells was determined in a liquid scintillation counter. Results plotted as dose concentration versus proliferation for inventive compounds nos. 3506, 3556, 3563, 3576, 3581, 3582 and 3584 are shown in FIGS. 6 (3506), 7 (3556), 8 (3563), 9 (3576), 10 (3581 and 3582) and 11 (3584). Respective concentrations of inventive compound (shown in the figures) were added two hours prior to Con A and IL-2 activation. Background counts were less than 200 cpm. The inventive compounds tested inhibited thymocyte proliferation and activation at relatively low concentrations with IC.sub.50 values ranging from 0.26 to 2.3 .mu.M (for compounds nos. 3576 and 3506, respectively).

EXAMPLE 8

This example illustrates a method for examining an effect of the inventive compounds, showing potential as cancer therapies, on normal cells. This assay has been used clinically to evaluate recovery of patients' marrow following chemotherapy or radiation. In this specific example, inventive compounds nos. 3563, 3576, 3581 and 3584 were less deleterious to normal cells as compared to known chemotherapeutic compounds in vitro, such as, for example, vinblastine, 5-fluorouracil, doxorubicin or cisplatinum.

Mouse bone marrow cells are useful in this assay because they produce colonies, which can later be counted, in culture. The colonies are called colony forming unit-granulocyte macrophage (CFU-GM) and depend on a source of colony stimulating factor for growth.

Mouse spleen conditioned-medium, at a concentration of 2%, was used in this assay. The medium and semi-solid culture mix were procured from Stem Cell Technologies in Vancouver, BC. In animal studies performed in a related cytoreductive treatment analysis, no CFU-GM were detectable in mouse femoral marrow during immediate days following 5-fluorouracil or treatment.

Cells were cultured with inventive compounds nos. 3563, 3576, 3581, 3584 and 4500, to comparatively evaluate an effect on normal cells of inventive compounds identified as potential cancer therapies. Procedurally, cells were incubated for 8 hours with various concentrations of these compounds. A negative control without any compound was simultaneously prepared. After 8 hours, the incubated cells were washed thoroughly and a consistent number were subsequently plated to obtain CFU-GM. Colonies were permitted to grow for 7 days at 37.degree. C., in 5% CO.sub.2. After 7 days of growth, colony growths were counted microscopically. Data obtained in this assay for inventive compounds nos. 3563, 3576, 3581, 3584 and 4500 are plotted in FIGS. 12, 13, 14, 15 and 16, respectively. The data in the figures are shown to compare the colonies counted for cells incubated with varying concentrations of the compounds tested against the negative control, without compound. At concentrations ranging from 5 to 30 .mu.M, more colonies existed for cells incubated with the inventive potential cancer compound than with known, chemotherapeutic agents. Compound no. 3563 was the most cytotoxic to proliferative potential of the mouse marrow cells, however, compound no. 4500 showed virtually no toxicity for the normal mouse marrow cells, as shown in FIG. 16.

These compounds tested exhibit potential as anti-cancer therapeutics. They are cytotoxic to cancer tumor cells. The results from this assay were used to predict whether these inventive compounds, which have anti-cancer potential, would be toxic to normal cells, such as the bone marrow cells representative of cells known to be adversely affected by known cancer therapies. As shown in these results, the inventive compounds are generally far less cytotoxic to normal bone marrow cells than known therapies and surprisingly, compound no. 4500 has virtually no toxicity at elevated concentrations for the normal mouse marrow cells. Such potential therapies which exhibit specific toxicity to tumor cells but are not cytotoxic to normal cells predicts remarkable treatment potential for cancers.

EXAMPLE 9

This example is an assay used to measure anti-viral activity of inventive compounds nos. 3556, 3563, 3576, 3580, 3581, 3582, 3584, 3590, 3593, 4500, 4507 and 4508, by evaluating the extent to which the compounds inhibit gene expression directed by specific viral promoters in cell lines. This assay is predictive of anti-viral activity for retroviruses. Specifically, a plasmid construct, pHIV.AP, using the human immunodeficiency virus (HIV) long terminal repeat (LTR) promotor [derived from pU3R-III CAT (Sodroski et al. Science, Vol. 227, page 171, 1985) to direct the expression of secreted human placental alkaline phosphatase reporter gene and an expression vector for a 72 amino acids tat protein from HIV (Frankel et al., Cell, Vol. 55, pages 1189-1193, 1988) were transfected into a tumor cell line (e.g., 293-EBNA cells). The stably transfected cells were treated with various concentrations of the inventive compounds. The expression of the alkaline phosphatase (AP) reporter gene in the individual cultures was then measured by following the change in absorbance at A405 of cell conditioned media in the presence of a suitable substrate (e.g., ortho-nitrophenol phosphate). Berger et al., Gene, Vol. 66, pages 1-10, 1988.

The effect of respective inventive compounds on the viability of 293-EBNA cells was measured by a calorimetric assay that uses the alamarBlue.RTM. dye (purchased from Alamar Biosciences, Inc.) to report cell proliferation, viability and cytotoxicity. This dye is an oxidation-reduction (Redox) indicator that changes color in response to chemical reduction of growth medium resulting from cell growth. The general procedure involves adding alamar-Blue.RTM. in an amount equal to 10% of the culture volume, returning the culture to incubator for four hours, and measuring the absorbance at 570 nm after subtraction of background absorbance at 600 nm.

Results obtained are shown in FIGS. 17, 18, 19, 20 and 21. FIG. 17 report data for inventive compounds nos. 3576, 3580, 3581, 3582, 3584 and 3590, showing the effect of these inventive compounds on expression of reporter gene directed by the HIV-LTR promotor in 293-EBNA cells. Compounds nos. 3581 and 3582 inhibited HIV-LTR expression by 50% (IC.sub.50) at <5 .mu.M; compounds nos. 3576, 3584, and 3580 at <10 .mu.M; and compound no. 3590 at <20 .mu.M. Corresponding cytotoxic effects of these compounds on 293-EBNA cells is shown in FIG. 18. Compounds nos. 3581 and 3582 had lethal dose 50% (LD.sub.50) values for 293-EBNA cells at <10 .mu.M and <5 .mu.M, respectively; compounds nos. 3576 and 3584 at <15 .mu.M; compound no. 3580 at <30 .mu.M; and compound no. 3590 had little cytotoxicity even at >32 .mu.M. Inventive compound no. 3580 inhibits HIV-LTR expression by 50% at <10 .mu.M and with minimal cytotoxicity. The compounds that inhibit 293-EBNA cell viability by 50% at <10 .mu.M show the greatest potential for use as an anti-cancer therapeutic also, as some of the inventive compounds exhibit tumor suppression activity.

FIG. 19 shows results obtained for inventive compounds nos. 3593, 3563, 4500, 4507 and 4508 in this assay. Compounds nos. 3563 and 4507 inhibited HIV-LTR expression by 50% (IC.sub.50) at <4 .mu.M; compounds nos. 4508 and 4500 at <10 .mu.M; and compound no. 3593 at <25 .mu.M. FIG. 20 illustrates corresponding

cytotoxic data of these compounds on 293-EBNA cells. Compounds nos. 3563 and 4507 have LD.sub.50 values of <7 .mu.M; compound no. 4508 at <10 .mu.M; compounds nos. 3593 and 4500 have little cytotoxicity, even at >32 .mu.M. Inventive compound no. 4500 inhibits HIV-LTR expression by 50% at <10 .mu.M yet has minimal cytotoxic effect. The compounds that inhibit 293-EBNA cell viability by 50% at <10 .mu.M also have the greatest potential for use as anti-cancer therapeutics.

FIG. 21 illustrates results obtained in this assay for inventive compound no. 3556, having an IC.sub.50 value of about 2.5 .mu.M using the HIV-LTR promotor construct cotransfected with a tat expression vector. Cytotoxicity of this compound is reported as the curve corresponding to alamarBlue.RTM.. Inventive compound no. 3556 has an LD.sub.50 value of around 12.5 .mu.M and cytotoxicity becomes significant for this compound at concentrations >10 .mu.M.

These assay data predict that the inventive compounds, as represented by those compounds tested, exhibit anti-viral activity, particularly against infection and viral replication of retroviruses, such as this HIV virus.

EXAMPLE 10

This example shows an ability of the inventive compounds, as represented by inventive compound no. 3556, to prevent promotion of fresh isolates of JR-CSF strain of HIV-1 infection of human peripheral blood lymphocytes (PBL).

In a protocol for infecting a PBL cell suspension, JR-CSF HIV-1 was clarified at low-speed centrifugation (2,000-3,000 rpm) or filtration and stored in aliquots at -70.degree. C. To maintain consistent titers, HIV-1 aliquots were not subjected to repeated freezing and thawing and any remainder was discarded. Virus aliquots were thawed at room temperature or under cold running water for more rapid thawing and maintained in ice after thawing. Just prior to infection of PBL cells, the supernatant or dilution were warmed to room temperature.

Human PBL cells taken from a suitable donor were centrifuged down at 1000 rpm for 5-10 minutes at room temperature. While cells were spinning, the virus inoculum was prepared in medium by adding 10 .mu.g/ml Polybrene.RTM.. The centrifuged cells were resuspended in virus inoculum, using 1 ml of virus inoculum per 10.sup.7 cells. A more efficient HIV-1 infection is obtained with smaller amounts of inoculum. The cells were incubated at 37.degree. C. in the presence of virus inoculum for 2 hours, shaking the suspension every half hour. After 2 hours, the suspension was spun down, the virus inoculum removed and the cells were washed with 10 ml of fresh media.

The infected cells, inoculated with virus at a concentration of 10 ng virus/10.sup.6 PBL, were washed and resuspended in growth medium (serum-free RPMI/20% FBS/10 units/ml IL-2). The washed and resuspended cells were added to each well of a 24-well plate at a concentration of approximately 10.sup.6 cells/well. The respective inventive compound was added to the wells at various concentrations, in triplicate. Supernatant from respective wells was harvested after days four and seven, 1 ml of fresh growth medium with antiretroviral agent was added and the samples collected were stored at -70.degree. C. On day fourteen, a final supernatant was harvested and p24 antigen ELISA analysis was performed on respective supernatant samples collected to determine whether the inventive compounds prevented viral infection.

The analysis results are graphically represented in FIG. 22 for inventive compound no. 3556. Inventive compound no. 3556 exhibited even more remarkable results in preventing infection. As shown in FIG. 22, at 1 .mu.M, compound no. 3556 decreased infection by about two to three fold, but at 10 and 20 .mu.M concentrations, compound no. 3556 virtually eliminated viral infection by this HIV-1 strain. At 20 .mu.M concentrations, some cell death began to occur at day seven. These results confirm that the inventive compounds, as represented by the tested specie, predict potent anti-viral activity against HIV and are thus effective therapeutics as anti-viral agents (particularly retroviruses) and for treating AIDS and AIDS-related indications.

COMPARATIVE EXAMPLE 1

This example illustrates the effects of comparative compounds A and B [1-(7-oxooctyl)-3,7-dimethylxanthine and 1-(5-dimethylaminohexyl)-3,7-dimethylxanthine, respectively] on inhibition of PDGF-induced proliferation in human stromal cells. Human stromal cells were starved in serum-free media for 24 hours and then stimulated with 50 ng/ml PDGF-BB. The drugs were added at various concentrations one hour prior to PDGF stimulation. Tritiated thymidine was added at the time of PDGF stimulation and pulsed for 24 hours. Cells were harvested and cell proliferation measured (FIG. 23). Background counts (i.e., starved cells) were about 10%

of control levels.

When compared with results obtained for the inventive compounds in a related assay using Balb/3T3 cells in place of human stromal cells (as shown in Example 5), these comparative compounds do not exhibit the significant therapeutic potential of the inventive compounds. The inventive compounds differ structurally from comparative compounds A and B at the amine substituents. The activity of the comparative compounds is apparent in millimolar concentrations, whereas the inventive compounds exhibit far more substantial activity in the micromolar range. Surprisingly, the inventive compounds have remarkably increased therapeutic potential over these comparative compounds.

* * * * *

US006316444B1

(12) **United States Patent**
Hunt et al.

(10) **Patent No.: US 6,316,444 B1**
(45) **Date of Patent: Nov. 13, 2001**

(54) **SRC KINASE INHIBITOR COMPOUNDS**

(75) Inventors: **Julianne A. Hunt; Sander G. Mills; Peter J. Sinclair; Dennis M. Zaller**, all of Scotch Plains, NJ (US)

(73) Assignee: **Merck & Co., Inc.**, Rahway, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 33 days.

(21) Appl. No.: **09/603,699**

(22) Filed: **Jun. 26, 2000**

Related U.S. Application Data

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(51) **Int. Cl.**[7] **A61P 37/06**; A61K 31/5377; A61K 31/506; C07D 401/14; C07D 403/14

(52) **U.S. Cl.** **514/231.2**; 514/234.2; 514/234.5; 514/235.8; 514/227.8; 514/228.5; 514/228.2; 514/249; 514/261; 514/275; 514/433; 540/598; 544/277; 544/278; 544/283; 544/284; 544/296; 544/310; 544/119; 544/117; 544/60; 544/61; 544/62; 544/350

(58) **Field of Search** 544/277, 278, 544/283, 284, 296, 310, 119, 117, 60, 61, 62, 350, 122; 540/598; 514/261, 275, 231.2, 234.2, 234.5, 235.8, 227.8, 228.5, 228.2, 433, 249

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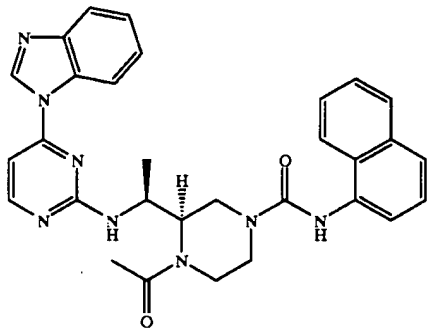
Primary Examiner—Mark L. Berch
Assistant Examiner—Kahsay Habte
(74) *Attorney, Agent, or Firm*—Valerie J. Camara; Mark R. Daniel

(57) **ABSTRACT**

Pyrimidine compounds (Formula I), or their pharmaceutically acceptable salts, hydrates, solvates, crystal forms and individual diastereomers, and pharmaceutical compositions including the same, which are inhibitors of tyrosine kinase enzymes, and as such are useful in the prophylaxis and treatment of protein tyrosine kinase-associated disorders, such as immune diseases, hyperproliferative disorders and other diseases in which inappropriate protein kinase action is believed to play a role, such as cancer, angiogensis, atherosclerosis, graft rejection, rheumatoid arthritis and psoriasis.

41 Claims, No Drawings

EXAMPLE 44

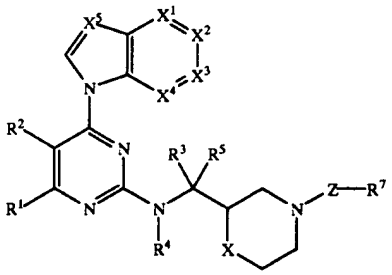


(S,S)2-[1(1-acetyl-4-(N-naphth-1-yl-carbamoyl)piperazine-2-yl)-ethylamino]-4-benzimidazol-1-yl]pyrimidine

To a solution of 2-[1-(4-(N-naphth-1-yl-carbamoyl)-piperazin-2-yl)ethylamino]-4-[benzimidazol-1-yl] pyrimidine (EXAMPLE 36, Step C; 19 mg) dissolved in 2 mL of CH_2Cl_2 and 0.5 mL of pyridine was added 7.9 mg of acetic anhydride. The solution was stirred for 30 minutes at room temperature, then diluted with 3 mL of ethyl acetate and extracted with 3 mL of saturated Na2HCO$_3$. The organic phase was concentrated and co-concentrated with 2×1.5 mL of heptane. The residue was purified by preparative thin-layer chromatography, eluting with 9:1 CH_3C1-isopropanol to provide 6.4 mg of the title compound. Mass spectrum (ESI) 535.5 (M+1).

What is claimed is:

1. A compound of Formula I



or pharmaceutically acceptable salts, hydrates, solvates, crystal forms and individual diastereomers thereof, wherein R^1 and R^2 are independently:

a) H,
b) halo(Br, Cl, I, or F),
c) OH,
d) SH,
e) CN,
f) NO_2,
g) R^9,
h) OR^9,
i) $O(C{=}O)R^9$,
j) $O(C{=}O)OR^9$,
k) $O(C{=}O)NHR^9$,
l) $O(C{=}O)NR^9R^{10}$,
m) SR^9,
n) $S(O)R^9$,
o) $S(O)_2R^9$,
p) $C(=O)R^9$,
q) $C(=O)OR^9$,
r) $C(=O)NHR^9$,
s) $C(=O)NR^9R^{10}$,
t) NH_2,
u) NHR^9,
v) NR^9R^{10},
w) $NHC(=O)R^9$,
x) $NHC(=O)OR^9$,
y) $NR^9C(=O)R^{10}$,
z) $NR^9C(=O)NHR^{10}$,
aa) $NR^9C(=O)NR^{10}R^{11}$,
ab) SO_2NHR^9,
ac) $SO_2NR^9R^{10}$,
ad) $NHSO_2R^9$,
ae) $NR^9SO_2R^{10}$, or
af) R^1 and R^2 can join together to form a fused methyl-enedioxy ring or a fused 6-membered aromatic ring;

R^3 and R^5 are independently:

a) H,
b) C_1–C_6-alkyl, unsubstituted or substituted with one, two, or three substituents selected from oxo, X', Y' and Z',
c) aryl, wherein aryl is defined as phenyl or naphthyl unsubstituted or substituted with one, two or three substituents selected from: X', Y' and Z', or
d) R^3 and R^5 taken together can represent =O;

R^4 is:

a) H, or
b) C_1–C_6-alkyl,
c) C_1–C_6-alkoxyl, or
d) R^4 and R^8 can join together to form a 5- or 6-membered ring with —CHR^9—, —CH_2CHR^9—, or —CHR^9CH_2—;

$—X^1—X^2—X^3—X^4—$ is:

a) $—CR^6{=}CR^6—CR^{6a}{=}CR^6—$,
b) $—CR^{6a}{=}CR^6—CR^6{=}CR^6—$,
c) $—CR^6{=}CR^{6a}—CR^6{=}CR^6—$,
d) $—CR^6{=}CR^6—CR^6{=}CR^{6a}—$,
e) $—N{=}CR^6—CR^6{=}CR^6—$,
f) $—CR^6{=}N—CR^6{=}CR^6—$,
g) $—CR^6{=}CR^6—N{=}CR^6—$,
h) $—CR^6{=}CR^6—CR^6{=}N—$,
i) $—N{=}CR^6—N{=}CR^6—$,
j) $—CR^6{=}N—CR^6{=}N—$,
k) $—CR^6{=}N—N{=}CR^6—$, or
l) $—N{=}CR^6—CR^6{=}N—$;

X^5 is N or CH;

R^6 and R^{6a} are independently:

a) H,
b) halo(Br, Cl, I, or F),
c) OH,
d) SH,
e) CN,
f) NO_2,
g) N_3,
h) $N_2{+}BF_4—$,
i) R^9,

US006335324B1

(12) **United States Patent**
Bisacchi et al.

(10) **Patent No.: US 6,335,324 B1**
(45) **Date of Patent: Jan. 1, 2002**

(54) **BETA LACTAM COMPOUNDS AND THEIR USE AS INHIBITORS OF TRYPTASE**

(75) Inventors: **Gregory S. Bisacchi**, Ringoes; **William A. Slusarchyk**, Skillman, both of NJ (US); **Uwe Treuner**, Yardley, PA (US); **James C. Sutton**, Princeton Junction, NJ (US); **Robert Zahler**; **Steven Seiler**, both of Pennington, NJ (US); **David R. Kronenthal**, Yardley, PA (US); **Michael E. Randazzo**, East Windsor, NJ (US); **Mark D. Schwinden**, Holland, PA (US); **Zhongmin Xu**, Plainsboro; **Zhongping Shi**, West Windsor, both of NJ (US)

(73) Assignee: **Bristol-Myers Squibb Co.**, Princeton, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(22) Filed: **Dec. 13, 1999**

Related U.S. Application Data

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(60) Provisional application No. 60/090,636, filed on Jun. 25, 1998.

(51) **Int. Cl.**[7] **C07D 40/14**; C07D 403/14; C07D 403/06; C07D 205/08; A61P 11/06

(52) **U.S. Cl.** **514/210.02**; 514/210.15; 540/200; 540/354; 540/355; 540/359; 540/362

(58) **Field of Search** 540/200, 354, 540/355, 359, 362; 514/210.02, 210.15

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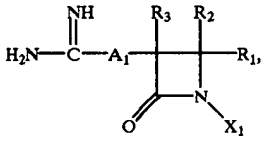
(List continued on next page.)

Primary Examiner—Mark L. Berch
(74) *Attorney, Agent, or Firm*—Stephen B. Davis

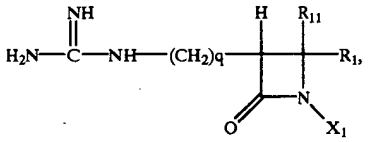
(57) **ABSTRACT**

Compounds of the formulas:

(I)



(II)



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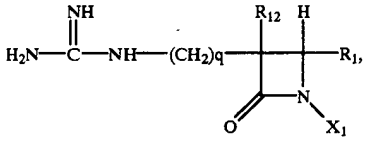

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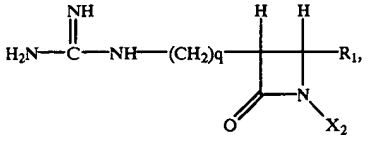

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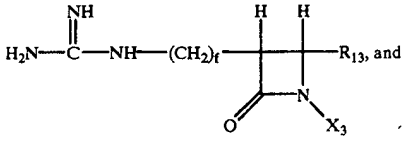
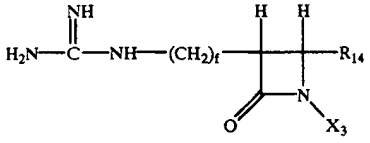


(VI)



are disclosed. These compounds inhibit tryptase as well as other enzyme systems or are selective tryptase inhibitors and are useful as antiinflammatory agents particularly in the treatment of chronic asthma.

39 Claims, No Drawings

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Baldwin et al., "Stereospecific Synthesis Of Dealanylalahopcin", Tetrahedron, vol. 46, Nos. 13, 14, p. 4733–48 (1990).

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Anderson et al., "Isoquinoline Derivatives as Local Anesthetics ", Journal American Pharmaceutical Assoc., vol. 41, No. 12, p. 643–650 (1952).

Sakai et al., "A Novel Heparin–dependent Processing Pathway For Human Tryptase", Journal Clinical Invest., vol. 97, No. 4, p. 988–95 (1996).

Han et., "Azetidine–2–one Derivatives as Inhibitors of Thromoin", Biorganic & Medicinal Chemistry, vol. 3, No. 8, p. 1123–43 (1995).

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activity (IC_{50}), the fraction of control activity (FCA) was plotted as a function of the inhibitor concentration (I) and curve to fit $FCA/(1+[I]/IC_{50})$. The IC_{50} for each compound was determined 2–4 times and the obtained values were averaged.

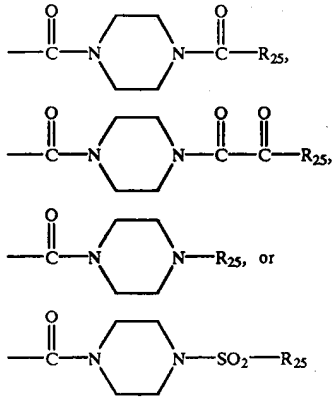
As a result of this tryptase activity, the compounds of formula I to VI as well as an inner salt thereof, a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof, are useful as antiinflammatory agents particularly in the treatment of chronic asthma and may also be useful in treating or preventing allergic rhinitis, inflammatory bowel disease, psoriasis, conjunctivitis, atopic dermatitis, rheumatoid arthritis, osteoarthritis, and other chronic inflammatory joint diseases, or diseases of joint cartilage destruction. Additionally, these compounds may be useful in treating or preventing myocardial infarction, stroke, angina and other consequences of atherosclerotic plaque rupture. Additionally, these compounds may be useful for treating or preventing diabetic retinopathy, tumor growth and other consequences of angiogenosis. Additionally, these compounds may be useful for treating or preventing fibrotic conditions, for example, fibrosis, scleroderma, pulmonary fibrosis, liver cirrhosis, myocardial fibrosis, neurofibromas and hypertrophic scars.

The compounds of formula I to VI are also inhibitors of Factor Xa and/or Factor VIIa. As a result, the compounds of formula I to VI as well as an inner salt or a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof may also be useful in the treatment or prevention of thrombotic events associated with coronary artery and cerebrovascular disease which include the formation and/or rupture of atherosclerotic plaques, venous or arterial thrombosis, coagulation syndromes, ischemia and angina (stable and unstable), deep vein thrombosis (DVT), disseminated intravascular coagulopathy, Kasacach-Merritt syndrome, pulmonary embolism, myocardial infarction, cerebral infarction, cerebral thrombosis,transient ischemic attacks, atriala fibrillation, cerebral embolism, thromboembolic complications of surgery (such as hip or knee replacement, introduction of artificial heart valves and endarterectomy) and peripheral arterial occulsion and may also be useful in treating or preventing myocardial infarction, stroke, angina and other consequences of atherosclerotic plaque rupture. The compounds of formula I to VI possessing Factor Xa and/or Factor VIIa inhibtion activity may also be useful as inhibitors of blood coagulation such as during the preparation, storage and fractionation of whole blood.

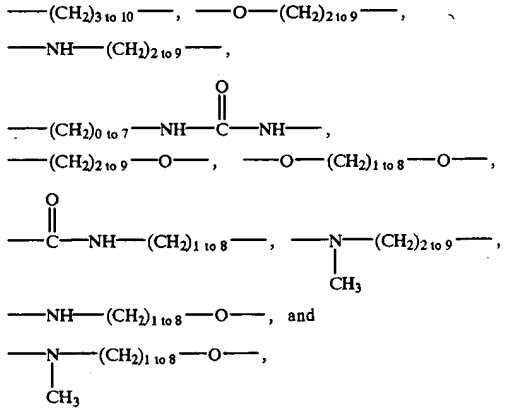
The compounds of formula I to VI are also inhibitors of urokinase-type plasminogen activator. As a result, the compounds of formula I to VI as well as an inner salt or a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof may be useful in the treatment or prevention of restenosis and aneurysms, in the treatment or prevention of myocardial infarction, stroke, angina and other consequences of atherosclerotic plaque rupture, and may also be useful in the treatment of malignancies, prevention of metastases, prevention of prothrombotic complications of cancer, and as an adjunct to chemotherapy.

The compounds of formulas I to V also possess thrombin and trypsin inhibitory activity similar to that reported by Han in the U.S. patents noted previously for the compounds of formula VI. As a result, the compounds of formula I to V as well as an inner salt or a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof may be useful in treating or preventing pancreatitis, in the treatment or prevention of thrombotic events associated with coronary artery and cerebrovascular disease as described above, and may also be useful as inhibitors of blood coagulation such as during the preparation, storage, and fractionation of whole blood.

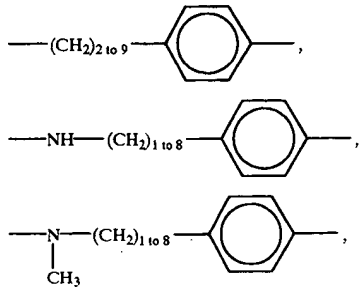
Certain compounds of formulas I to IV are also useful due to their selective tryptase inhibition activity. These compounds while having potent tryptase inhibition activity are much less active against other enzyme systems including trypsin, thrombin and Factor Xa. For example, this selective tryptase activity is seen with the compounds of formulas I to IV where X_1 or X_2 is the group



and R_{25} is a spacer terminating in a lipophilic group. Suitable spacers include groups of 3 or more atoms such as



etc., as well as groups containing 2 or more atoms and a phenyl, substituted phenyl, cycloalkyl, heteroaryl, or heterocycloalkyl ring such as



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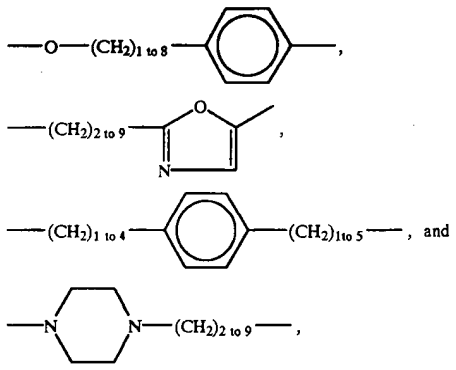


etc. Suitable lipophilic terminal groups include aryl, substituted aryl, cycloalkyl, heteroaryl, heterocycloalkyl, etc. These compounds of formulas I to IV as well as an inner salt, a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof, are useful as antiinflammatory agents particularly in the treatment of chronic asthma and may also be useful in treating or preventing allergic rhinitis as well as some of the other diseases described above for the non-selective tryptase inhibitors. It is believed that as a result of their selective tryptase inhibition activity that these compounds will have less tendency to produce unwanted side-effects.

The compounds of formula I to VI as well as an inner salt or a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof may be administered orally, topically, rectally or parenterally or may be administered by inhalation into the bronchioles or nasal passages. The method of administration will, or course, vary upon the type of disease being treated. The amount of active compound administered will also vary according to the method of administration and the disease being treated. An effective amount will be within the dosage range of about 0.1 to about 100 mg/kg, preferably about 0.2 to about 50 mg/kg and more preferably about 0.5 to about 25 mg/kg per day in a single or multiple doses administered at appropriate intervals throughout the day.

The composition used in these therapies can be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, liposomes, injectable and infusible solutions. Such compositions can include pharmaceutically acceptable carriers, preservatives, stabilizers, and other agents conventionally employed in the pharmaceutical industry.

When the compounds of formula I to VI as well as an inner salt or a pharmaceutically acceptable salt thereof, a hydrolyzable ester thereof, or a solvate thereof are employed to treat asthma or allergic rhinitis they will preferably be formulated as aerosols. The term "aerosol" includes any gas-borne suspended phase of the active compound which is capable of being inhaled into the bronchioles or nasal passage. Aerosol formulations include a gas-borne suspension of droplets of the active compound as produced in a metered dose inhaler or nebulizer or in a mist sprayer. Aerosol formulations also include a dry powder composition suspended in air or other carrier gas. The solutions of the active compounds of formulas I to VI used to make the aerosol formulation will be in a concentration of from about 0.1 to about 100 mg/ml, more preferably 0.1 to about 30 mg/ml, and most preferably from about 1 to about 10 mg/ml. The solution will usually include a pharmaceutically acceptable buffer such as a phosphate or bicarbonate to give a pH of from about 5 to 9, preferably 6.5 to 7.8, and more preferably 7.0 to 7.6. Preservatives and other agents can be included according to conventional pharmaceutical practice.

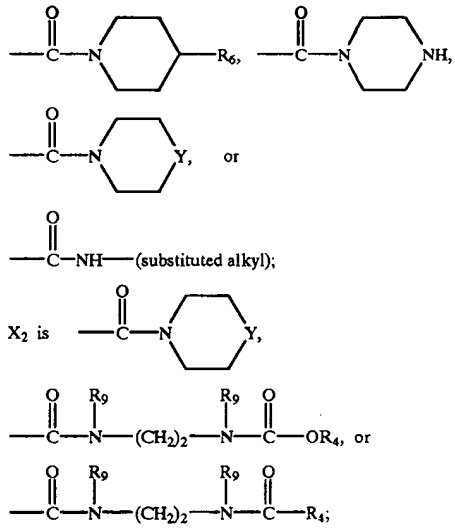
Other pharmaceutically active agents can be employed in combination with the compounds of formula I to VI depending upon the disease being treated. For example, in the treatment of asthma, β-adrenergic agonists such as albuterol, terbutaline, formoterol, fenoterol or prenaline can be included as can anticholinergics such as ipratropium bromide, anti-inflammatory cortiocosteroids such as beclomethasone, triamcinolone, flurisolide or dexamethasone, and anti-inflammatory agents such as cromolyn and nedocromil.

In addition to the novel compounds of formulas I to V and the methods of use for the compounds of formulas I to VI, this invention is also directed to novel intermediates and novel synthetic routes employed in the preparation of such compounds.

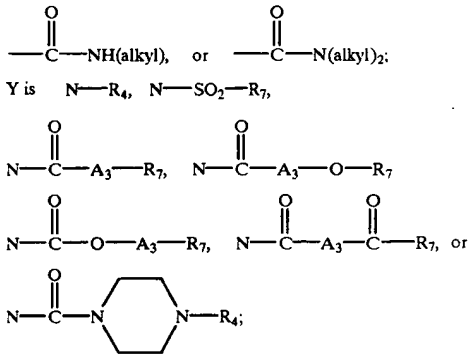
Preferred compounds of this invention are those of formula IV wherein:

q is 3;

R_1 is carboxy,



R_6 is aminocarbonyl,



R_4 in the definition of Y and X_2 is alkyl, cycloalkyl, substituted alkyl, substituted cycloalkyl, $-(CH_2)_{1\ to\ 6}$-aryl, or heteroaryl;

R_7 is alkyl, cycloalkyl, substituted alkyl, substituted cycloalkyl, $-(CH_2)_{0\ to\ 4}$-aryl, $-(CH_2)_{0\ to\ 4}$-aryl-A_3-

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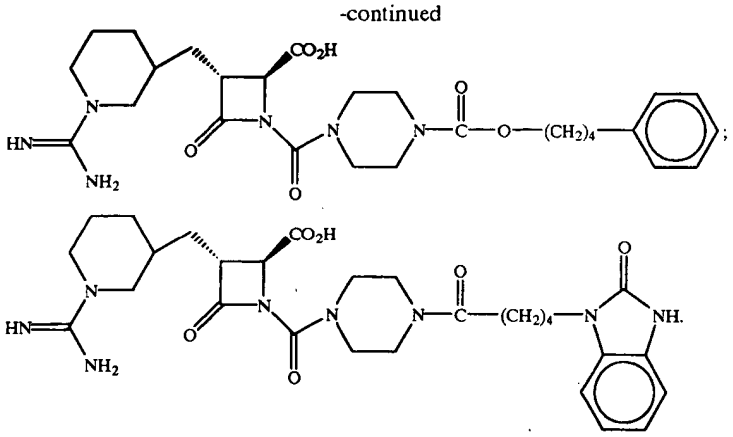
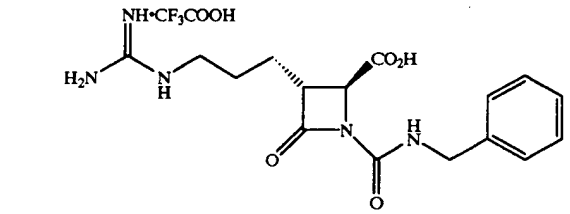


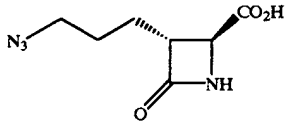
The following examples are illustrative of the invention.

EXAMPLE 1

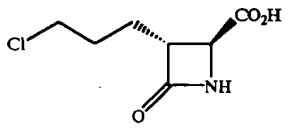
(homochiral)



a)



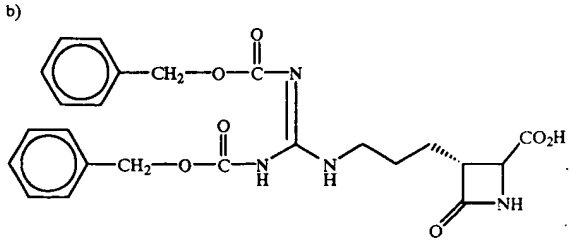
n-Butyl lithium in hexanes (2.5 M, 36.6 ml, 91.5 mmol) was added dropwise over 5 minutes to a solution of diisopropylamine (13.5 ml, 95.9 mmol) in dry tetrahydrofuran (40 ml) under nitrogen at −78° C. with mechanical stirring. After warming to 0° C. and stirring for 30 minutes, the solution was cooled to −78° C. and (4S)-N-(t-butyldimethylsilyl)-azetidine-2-one-4-carboxylic acid (10 g, 43.6 mmol) [Baldwin et al, Tetrahedron, Vol. 46, p. 4733–4748, 1990] was added in a single portion. After stirring the reaction mixture—gelatinous suspension at −78° C. for 5 minutes, the reaction was warmed to −20° C. to −10° C. and stirred at this temperature for 30 minutes. 1-Chloro-3-iodopropane (5.7 ml, 53.0 mmol) was added in a single portion and the reaction was stirred at −20° C. for 2 hours (gelatinous suspension disappears upon the addition of 1-chloro-3-iodopropane). The reaction mixture was then poured into 1N HCl saturated with sodium chloride (300 ml) and the aqueous phase was extracted with ethyl acetate (1×150 ml) which was then washed twice with saturated 1N HCl. The aqueous layers were then extracted twice, in order, with ethyl acetate (2×150 ml). The combined organics were then extracted twice with pH 7.5–8 water (2×100 ml, adjusted by the dropwise addition of 25% sodium hydroxide). The combined basic aqueous layers were then washed with ethyl acetate (2×150 ml). The basic aqueous layers were then acidified with concentrated HCl to pH 3, saturated with sodium chloride (solid) and extracted with ethyl acetate (2×150 ml), dried over sodium sulfate, filtered and concentrated. Evaporative drying with toluene then gave:



as a crude yellow oil. TLC (silica gel, 1% acetic acid in ethyl acetate) R_f=0.1, streaks.

Tetrabutylammonium iodide (0.5 g, 1.36 mmol) and tetrabutylammonium azide (15 g, 52.7 mmol) were added to a solution of the crude chloride from above (less than 43.6 mmol) in dry dimethylformamide (40 ml) under nitrogen. After stirring the reaction mixture at room temperature for 72 hours, the majority of the dimethylformamide was removed under vacuum. The product was extracted into ethyl acetate (150 ml) which was washed with 1N HCl saturated with sodium chloride (3×150 ml). The aqueous layers were extracted, in order, with ethyl acetate (2×150 ml). The combined organics were then extracted into pH 7.5–8 water (2×100 ml, adjusted by the dropwise addition of 25% sodium hydroxide). The combined basic aqueous layers were then washed with ethyl acetate (3×150 ml). The basic aqueous layers were then acidified with concentrated HCl to pH 3, saturated with sodium chloride (solid) and extracted with ethyl acetate, dried over sodium sulfate, filtered and concentrated. Evaporative drying with toluene gave the desired azide as a yellow-brown foam (6.56 g, 33.1 mmol). TLC (silica gel, 1% acetic acid in ethyl acetate) R_f=0.1

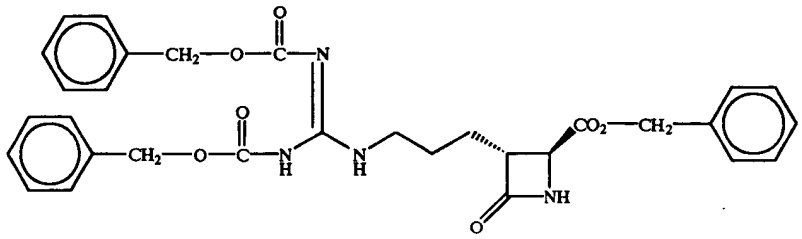
b)



Acetic acid (4 ml, 66 mmol) was added to a solution of the azide from step (a) (6.5 g, 32.8 mmol) in dimethylformamide (40 ml) followed by 10% palladium on carbon (1.3 g).

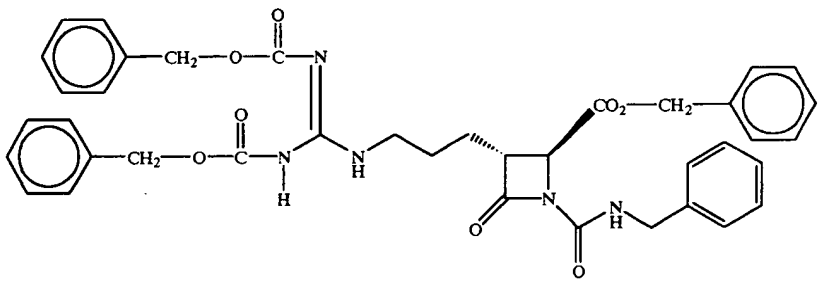
Hydrogen was bubbled through the reaction mixture for 25 minutes and then the reaction was stirred under hydrogen for 6 hours. After degassing the mixture with nitrogen for 25 minutes, triethylamine (15 ml, 108 mmol) and N,N'-bis (benzyl-oxycarbony)-1-guanylpyrazole (15 g, 39.7 mmol) [Wu et al., Synthetic Communications, 23(21), p. 3055–3060, (1993)] were added. The reaction was then stirred at room temperature for 18 hours. The reaction mixture was then filtered through Celite® which was then washed with ethyl acetate. Solvents were reduced under vacuum and the resulting residue was dissolved in ethyl acetate (150 ml) and washed with 1N HCl saturated with sodium chloride (3×150 ml). The aqueous washes were extracted, in order, with ethyl acetate (2×100 ml). The combined organics were then extracted with pH 7.5–8 water (2×150 ml, adjusted by the dropwise addition of 25% sodium hydroxide). The combined basic aqueous layers were then washed with ethyl acetate (3×150 ml) to remove excess N,N'-bis(benzyloxycarbonyl)-1-guanylprazole from the product. The basic aqueous layers were then acidified with concentrated HCl to pH 3, saturated with sodium chloride (solid), extracted with ethyl acetate (2×150 ml), dried over sodium sulfate, filtered and concentrated. Evaporative drying with toluene gave a light brown foam. Purification by flash chromatography (silica gel, 1–3% acetic acid in ethyl acetate) gave the desired product (8.5 g, 17.6 mmol) as an off-white solid. TLC (silica gel, 1% acetic acid in ethyl acetate) R_f=0.2.

c)



Solid sodium bicarbonate (1.5 g, 17.9 mmol), tetrabutylammonium iodide (200 mg, 0.54 mmol) and lastly benzyl bromide (2.5 ml, 21.0 mmol) were added to a solution of the product from step (b) (1.7 g, 3.52 mmol) in dimethylformamide (20 ml) under nitrogen at room temperature. The reaction mixture was stirred at room temperature for 48 hours. Dimethylformamide was removed under vacuum and the resulting residue was dissolved in ethyl acetate which was then washed twice with saturated aqueous sodium bicarbonate. The organic phase was separated, dried over magnesium sulfate, filtered and reduced to leave a brown oil. Purification by flash chromatography (silica gel, 0–10% methanol in methylene chloride) provided the desired product (1.84, 3.21 mmol) as a yellow oil.

d)

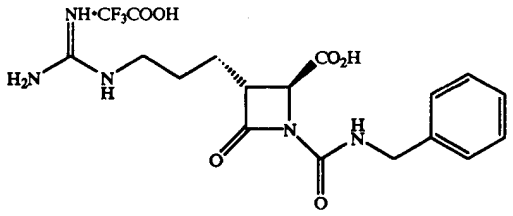


Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 100 μl, 0.1 mmol) was added to a solution of the product from step (c) (46 mg, 0.08 mmol) in dry tetrahydrofuran (1.0 ml) under nitrogen at −78° C. The reaction mixture was stirred at −78° C. for 10 minutes and then at −20° C. for 10 minutes. After cooling the reaction mixture to −78° C., benzyl isocyanate (100 μl, 0.78 mmol) was added in a single portion. The reaction mixture was

stirred at −78° C. for 5 minutes and then at −20° C. for 15 minutes. 1N HCl (1 ml) was added followed immediately by ethyl acetate (3 ml). The resulting biphasic solution was stirred vigorously while warming to room temperature. The organic phase was separated and washed once with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, filtered and concentrated to leave a light yellow residue. Purification by flash chromatography (silica gel, 0–30% ethyl acetate in hexane) gave the desired product (33 mg, 0.047 mmol).

e)

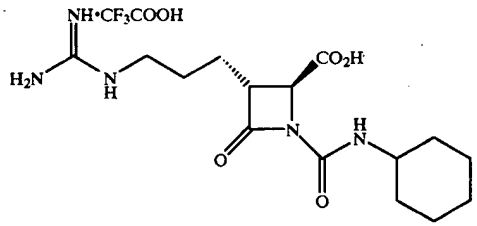
(homochiral)



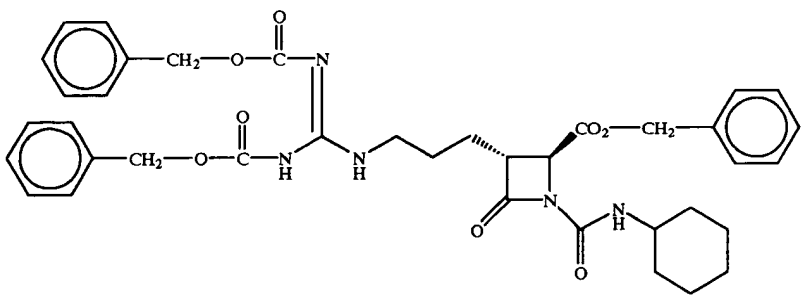
Concentrated HCl (4 μl, 0.048 mmol) was added to a solution of the product from step (d) (33 mg, 0.047 mmol) in dioxane (2 ml) followed by 10% palladium on carbon catalyst (15 mg). Hydrogen gas was bubbled through the reaction mixture for 1.5 hours. Water (0.5 ml) was added and the reaction was stirred under hydrogen for an additional 1 hour. The reaction mixture was then filtered through Celite® which was then washed with three portions of water. The combined eluent was lyophilized to give white powder. Purification by preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid) provided after lyophilization the desired product (8.7 mg, 0.019 mmol). IR (film) 1773 cm^{-1}; MS 348.0 $(M+H)^+$, 346.3 $(M-H)^-$.

EXAMPLE 2

(homochiral)



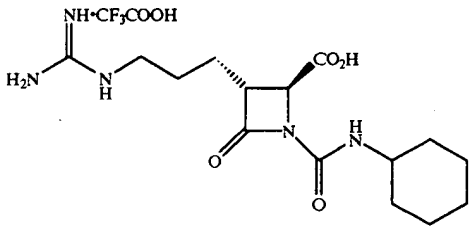
a)



Sodium hydride (60% in mineral oil, 20 mg, 0.5 mmol) was added to a solution of the product from Example 1(c) (92 mg, 0.161 mmol) in dry tetrayhydrofuran (1.5 ml) under nitrogen at room temperature. After stirring the reaction mixture for 10 minutes, cyclohexyl isocyanate (100 μl, 0.78 mmol) was added in a single portion. The reaction mixture was stirred at room temperature for 30 minutes. The reaction was then slowly poured over ice cold 1N HCl (2.5 ml). The resulting solution was extracted with ethyl acetate. The organic phase was washed twice with saturated aqueous sodium bicarbonate and once with brine. The organic layer was dried over sodium sulfate, filtered and concentrated. Purification by flash chromatography (silica gel, 0–10% methanol in methylene chloride) provided the desired product (91 mg, 0.13 mmol). MS 698.1 $(M+H)^+$, 696.4 $(M-H)^-$.

b)

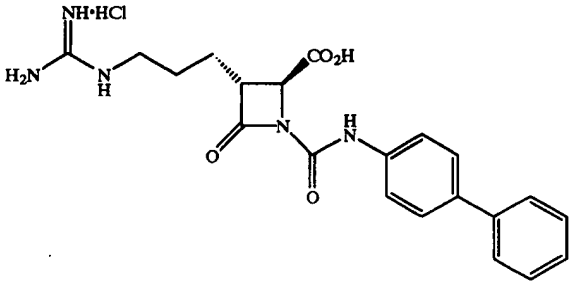
(homochiral)



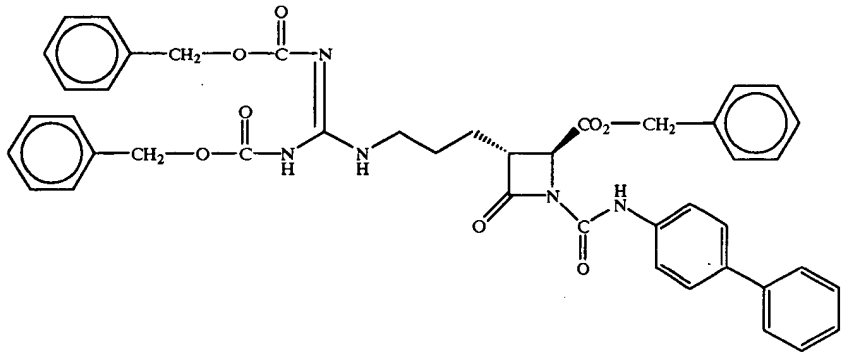
Concentrated HCl (11 μl, 0.132 mmol) was added to a solution of the product from step (a) (91 mg, 0.13 mol) in dioxane (2 ml) followed by 10% palladium on carbon catalyst (45 mg). H_2 was bubbled through the reaction mixture for 45 minutes. Water (1 ml) was added and the reaction was stirred under hydrogen for an additional 45 minutes. The reaction mixture was then filtered through Celite® which was then washed with three portions of water. The combined eluent was lyophilized to give white powder. Purification by preparative HPLC (reverse phase, methanol water, trifluoroacetic acid) provided after lyophilization the desired product (28 mg, 0.062 mmol). IR (KBr) 1773 cm^{-1}; MS 340.1 $(M+H)^+$, 338.2 $(M-H)^-$.

EXAMPLE 3

(homochiral)



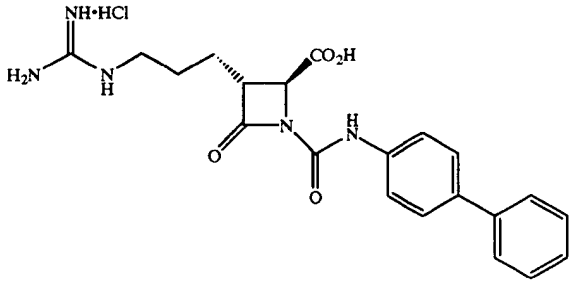
a)



Following the procedure of Example 1(d) but substituting 4-biphenylisocyanate for the benzyl isocyanate, the desired product was obtained. IR (film) 1776 cm^{-1}; MS 768.1 $(M+H)^+$, 766.2 $(M-H)^-$.

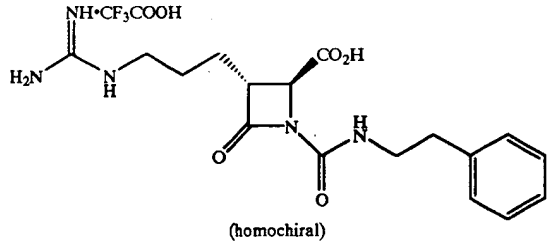
b)

(homochiral)



Concentrated HCl (10 μl, 0.12 mmol) was added to a solution of the product from step (a) (30 mg, 0.039 mmol) in dioxane (4 ml) followed by 10% palladium on carbon catalyst (30 mg). H_2 was bubbled through the reaction mixture for 5 minutes and then the reaction mixture was stirred under hydrogen gas for 1.5 hours. The reaction mixture was then filtered through Celite® which was then washed with two portions of water and one portion of dioxane. The combined eluent was lyophilized to give the desired product (16.3 mg, 0.036 mmol). IR (film) 1769 cm^{-1}; MS 410.1 $(M+H)^+$, 408.3 $(M-H)^-$.

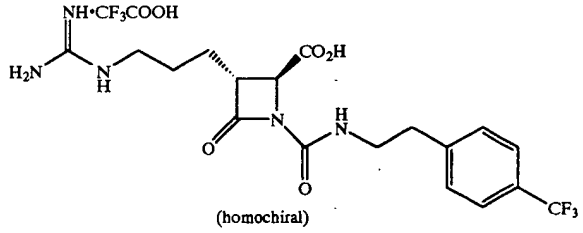
EXAMPLE 4



(homochiral)

Following the procedure of Example 1 but substituting phenethyl isocyanate for the benzyl isocyanate in step (d) followed by the deprotection work-up described in Example 1 step (e), the desired product was obtained. IR (film) 1775 cm^{-1}; MS 362.1 $(M+H)^+$, 360.3 $(M-H)^-$.

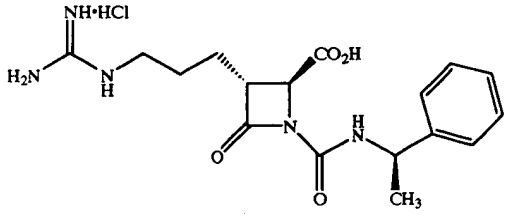
EXAMPLE 5



(homochiral)

Following the procedure of Example 1 but substituting 4-trifluoromethylphenyl isocyanate for the benzylisocyanate in step (d) followed by the work up described in Example 1 step (e), the desired product was obtained. IR (film) 1761 cm^{-1}; MS 402.1 $(M+H)^+$, 400.2 $(M-H)^-$.

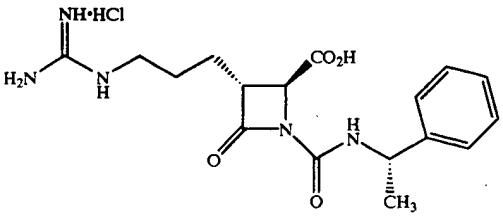
EXAMPLE 6



(homochiral)

Following the procedure of Example 1 but substituting (R)-α-methylbenzyl isocyanate for the benzyl isocyanate in step (d) followed by the deprotection and the work-up described in Example 3 (b), the desired product was obtained. IR (KBr) 1777 cm^{-1}; MS 362.1 $(M+H)^+$, 360.2 $(M-H)^-$.

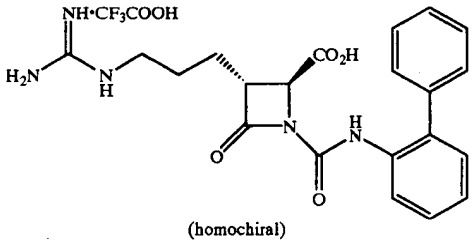
EXAMPLE 7



(homochiral)

Following the procedure of Example 1 but substituting (S)-α-methylbenzyl isocyanate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 3(b), the desired product was obtained. IR (KBr) 1777 cm^{-1}; MS 362.1 $(M+H)^+$, 360.3 $(M-H)^-$.

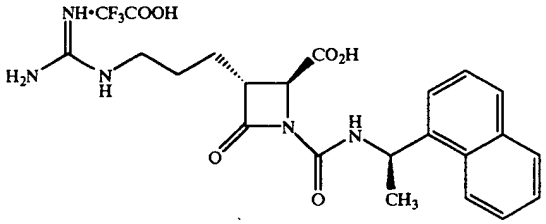
EXAMPLE 8



(homochiral)

Following the procedure of Example 1 but substituting 2-biphenyl isocyanate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 1(e), the desired product was obtained. IR (KBr) 1780 cm^{-1}; MS 410.1 $(M+H)^+$, 408.2 $(M-H)^-$.

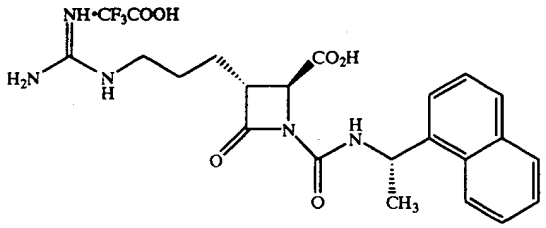
EXAMPLE 9



(homochiral)

Following the procedure of Example 1 but substituting (R)-(-)—(1-naphthyl)ethyl isocyanate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 1(e), the desired product was obtained. IR (KBr) 1777 cm^{-}, MS 412.3 $(M+H)^+$, 410.2 $(M-H)^-$.

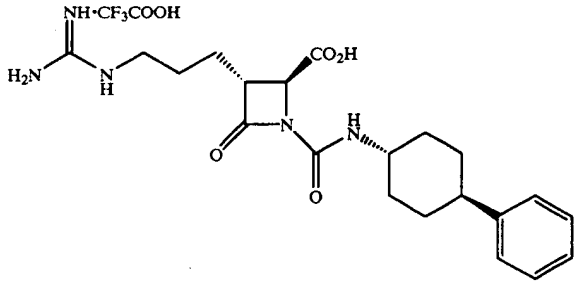
EXAMPLE 10



(homochiral)

Following the procedure of Example 1 but substituting (S)-(+)-(1-naphthyl)ethyl isocyanate for the benzyl isocyanate in step (d) followed by the work-up described in Example 1(e), the desired product was obtained. IR (KBr) 1777 cm^{-1}; MS 412.3 $(M+H)^+$, 410.2 $(M-H)^-$.

EXAMPLE 11



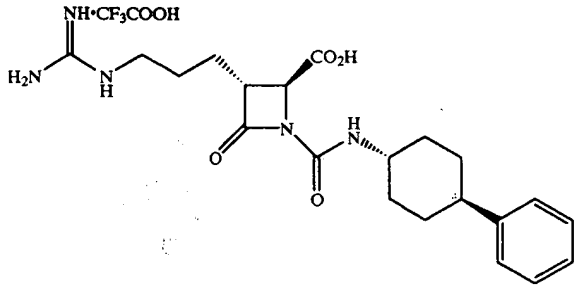
(homochiral)

a) trans-4-phenylcyclohexylisocyanate

Ammonium formate (18 g, 285 mmol) was added to a solution of 4-phenylcyclohexanone (5 g, 28.7 mmol) in methanol (150 ml) under nitrogen at room temperature followed by the portionwise addition of sodium cyanoborohydride (1.85 g, 29.4 mmol). After stirring the reaction mixture at room temperature for 24 hours, the methanol was removed under vacuum to leave an oily residue. The residue was dissolved in methylene chloride (100 ml) which was then washed with 1N sodium hydroxide (2×100 ml). The organic layer was separated, dried over sodium sulfate, filtered and concentrated to leave an off-white solid. Purification by flash chromatography (silica gel, 0–20% methanol/methylene chloride) provided 3.46 g of trans-4-phenyl-cyclohexylamine as a white solid.

Phosgene (20% in toluene, 5 ml) was added to a solution of trans-4-phenylcyclohexylamine (500 mg, 2.85 mmol) in toluene (5 ml) under nitrogen at room temperature. The resulting solution was heated at 80° C. for 24 hours. Solvents were then removed under vacuum to leave a solid residue. This residue was dispersed in ether and filtered. The eluent was collected and concentrated to give 379 mg of trans-4-phenylcyclohexylisocyanate as a light yellow oil. IR (film) 2259 cm^{-1}.

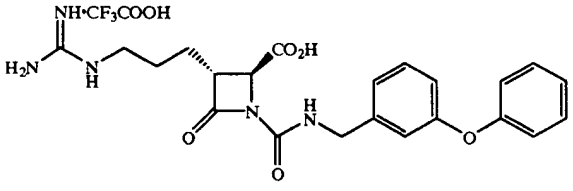
b)



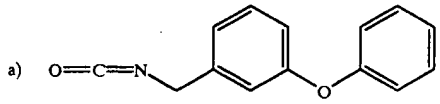
(homochiral)

Following the procedure of Example 1 but substituting trans-4-phenylcyclohexylisocyanate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 1(e), the desired product was obtained. IR (KBr) 1778 cm^{-1}; MS 416.2 $(M+H)^+$, 414.4 $(M-H)^-$.

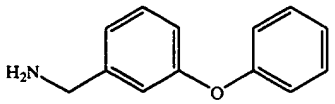
EXAMPLE 12



(homochiral)



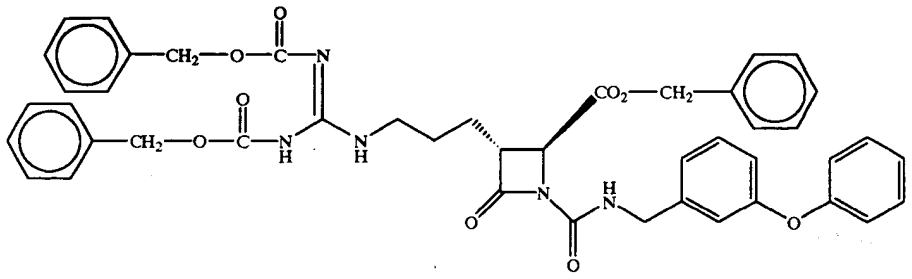
Ammonium formate (7.95 g, 126.12 mmol) and sodium cyanoborohydride (4.75 g, 75.66 mmol) were added to a solution of 3-phenoxybenzaldehyde (5g, 25.22 mmol) in methanol (125 ml) and the mixture was stirred at room temperature overnight. After 16 hours, the mixture was evaporated in vacuo and partitioned between 1N HCl and ethyl acetate. The aqueous layer was then basified using 6N sodium hydroxide solution to pH 12 and re-extracted with ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate and concentrated to give 300 mg of



as a colorless oil. MS 199.2 $(M+H)^+$.

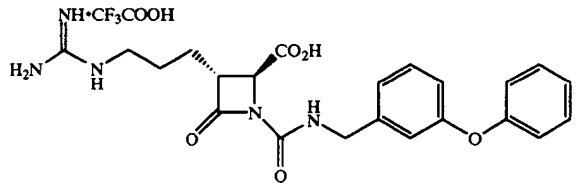
This amino compound (300 mg, 1.51 mmol) in toluene (2 ml) was added to a mixture of phosgene (3 ml of a 20% phosgene in toluene solution) in toluene (2 ml). The mixture was heated at 80° C. for 2 hours, followed by stirring at 110° C. for 1 hour and stirring at 80° C. overnight. The mixture was then evaporated in vacuo and the residue was suspended in ether and filtered. The eluents were concentrated and co-evaporated with toluene to give the desired isocyanate as a brown oil (0.328 g). IR (film) 2263.5 cm^{-1}.

b)



The benzyl ester product from Example 1(c) (69 mg, 0.121 mmol) was dissolved in tetrahydrofuran (1.5 ml) and cooled to -78° C. Sodium bis(trimethylsilyl)amide (0.15 ml, 0.145 mmol) was added over 2 minutes and the mixture was stirred at -78° C. for 1 hour. A solution of the isocyanate from step (a) (0.328 g, 0.145 mmol) in tetrahydrofuran (1.2 ml) was added over 1 minute and the reaction mixture was stirred at -78° C. After 30 minutes, the mixture was quenched with 0.5 N potassium bisulfate solution (10 ml) and extracted with ethyl acetate (2×10 ml). The organic phase was washed with brine (1×15 ml), dried over sodium sulfate, and condensed to give a yellow oil (150 mg). Purification by flash chromatography (silica gel, 0–25% ethyl acetate/hexane) gave the desired product as a pale yellow oil (50 mg). MS 798.1 $(M+H)^+$, 796.3 $(M-H)^-$; IR (film) 1776.6 cm^{-1}.

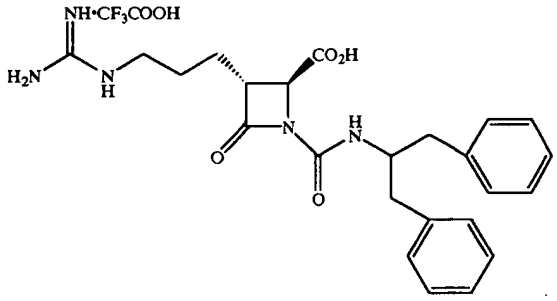
c)



(homochiral)

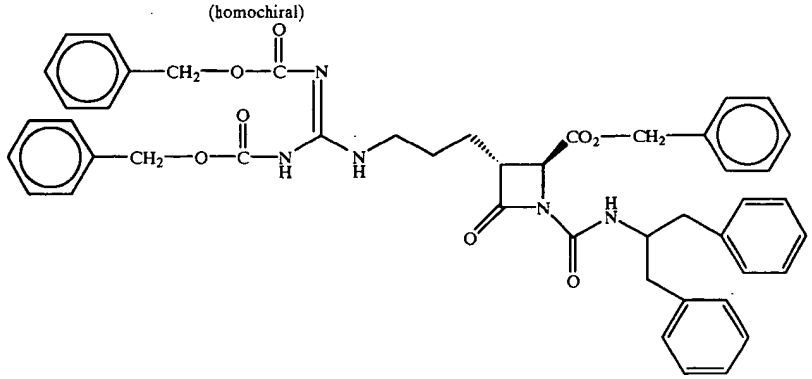
10% Palladium on carbon catalyst (25 mg, wet type) was added to a solution of the product from step (b) (45 mg, 0.056 mmol) in 1,4-dioxane (7 ml) containing 1N HCl. Hydrogen gas was bubbled through the solution for 4 hours. The resulting mixture was filtered through Celite® which was then repeatedly washed with 1,4-dioxane. The combined eluents were evaporated in vacuo to give a pale yellow glue (40 mg). Purification by reverse phase preparative HPLC (YMC ODS 30×250 mm) using the solvent system described in Example 1(e) gave the desired product as a white solid (26 mg). MS 440.2 $(M+H)^+$, 438.3 $(M-H)^-$; IR (KBr) 1773 cm^{-1}.

EXAMPLE 13



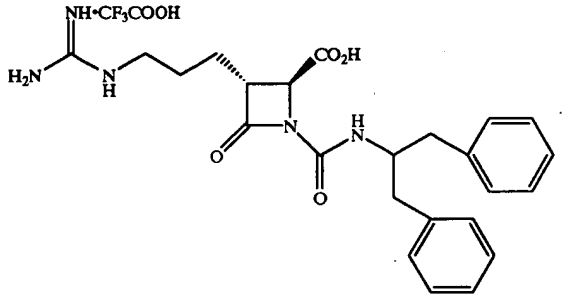
(homochiral)

a)



Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 106 μl, 0.106 mmol) was added to a solution of the benzyl ester product from Example 1(c) (57.9 mg, 0.101 mmol) in dry tetrahydrofuran (2 ml) under nitrogen at −78° C. The reaction mixture was stirred at −78° C. for 1 hour and then 1-benzyl-2-phenethyl isocyanate (35 mg, 0.147 mmol) [prepared as described by Anderson et al., J. American Pharm. Assoc., Vol. 41, p. 643–650 (1952)] was added in a single portion. The reaction mixture was stirred at −78° C. for 20 minutes. The reaction was quenched by the addition of potassium bisulfate (3 ml) followed immediately by the addition of ethyl acetate (5 ml). The resulting biphasic solution was stirred vigorously while warming to room temperature. The organic phase was separated, dried over sodium sulfate, filtered and concentrated to leave a bright yellow residue. Purification by flash chromatography (silica gel, 0–20% ethyl acetate in hexane) gave the desired product (54.8 mg, 0.068 mmol). IR (film) 1776 cm^{-1}; MS 810.2 $(M+H)^+$, 808.4 $(M-H)^-$.

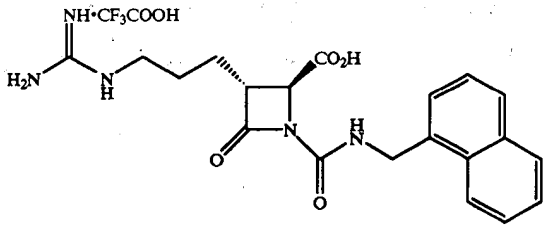
b)



(homochiral)

Concentrated HCl (20 μl, 0.24 mmol) was added to a solution of the product from step (a) (54.8 mg, 0.068 mmol) in dioxane (3 ml) followed by 10% palladium on carbon catalyst (50 mg). Hydrogen gas was bubbled through the reaction mixture for 5 minutes and then the reaction mixture was stirred under hydrogen gas for 1.5 hours. The reaction mixture was then filtered through Celite® which was then washed with two portions of dioxane and three portions of water. The combined eluent was lyophilized to give a white powder. Purification by preparative HPLC (reverse phase, methanol, water trifluoroacetic acid) provided after lyophilization the desired product (21 mg). IR (KBr) 1776 cm^{-1}; MS 452.4 $(M+H)^+$, 450.4 $(M-H)^-$.

EXAMPLE 14

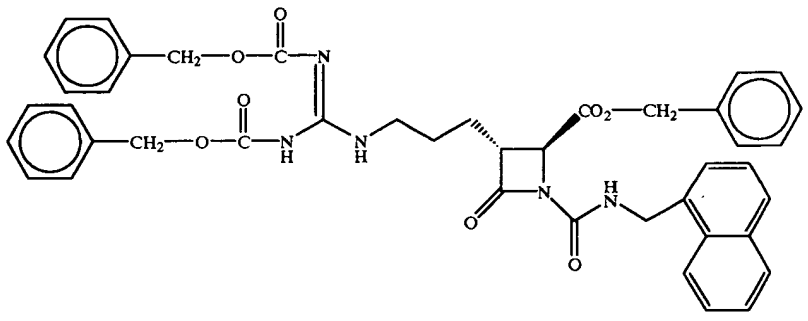


(homochiral)

a) 1-Naphthylmethylisocyanate

A solution of phosgene (20% in toluene, 5 ml) was diluted with toluene (10 ml). A mixture of 1-naphthalenemethylamine (500 μl, 3.41 mmol), triethylamine (0.95 ml, 6.82 mmol) in toluene (5 ml) was added dropwise. The reaction mixture was heated at reflux overnight. The mixture was cooled to room temperature, and the solvent was removed. The residue was stirred with ether (50 ml) for 10 minutes and filtered. The filtrate was concentrated to give the crude product which was purified by flash chromatography (silica gel, methylene chloride) to give the desired product (518 mg) as a colorless oil. IR 2260 cm^{-1}.

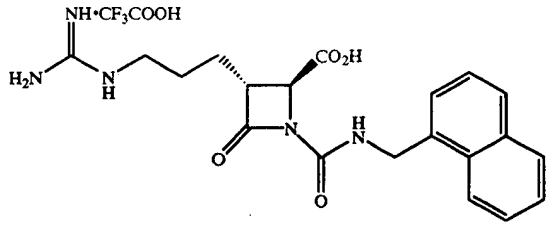
b)



Sodium bis (trimethylsilyl)amide (1.0 M in tetrahydrofuran, 300 μl, 0.30 mmol) was added dropwise to a −78° C. solution of the benzyl ester product from Example 1(c) (144 mg, 0.25) in tetrahydrofuran (3 ml). The mixture was stirred at −78° C. for 1 hour. A solution of 1-naphthylmethyl-isocyanate (55 mg, 0.30 mmol) in tetrahydrofuran (1 ml) was added. The reaction mixture was stirred at −78° C. for an additional 40 minutes. The reaction was quenched by the addition of 1N potassium bisulfate (15

ml). The mixture was extracted with ethyl acetate (2×40 ml). The organic layers were combined and washed with brine (15 ml), dried over magnesium sulfate, filtered and concentrated to give 185 mg of crude product as a yellow oil. Purification by chromatography (silica, 30–50% ethyl acetate/hexane) gave the desired product as a colorless oil (122 mg). MS: $(M+H)^+$ 756.1; IR (KBr) 1776 cm^{-1}, 1732 cm^{-1}, 1639 cm^{-1}.

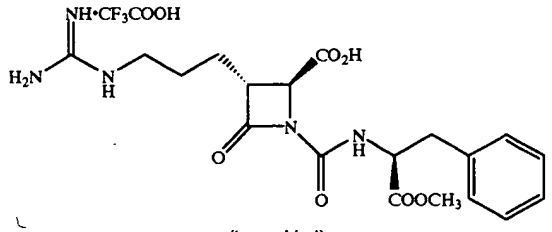
c)



(homochiral)

A mixture of the product from step (b) (117 mg, 0.15 mmol), 1N HCl (170 μl, 0.17 mmol), palladium on carbon catalyst (10%, 50 mg) in dioxane (3 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 1 hour. Analytical HPLC indicated the completion of the reaction. The reaction mixture was filtered through a Celite® cake and concentrated to give the crude product (68 mg) which was purified by reverse phase preparative HPLC as described in Example 1(e) to yield the desired product (37 mg) as a white powder. MS $(M+H)^+$ 398.2, $(M-H)^-$396.4; IR (KBr) 1780 cm^{-1}, 1670 cm^{-1}, 1541 cm^{-1}.

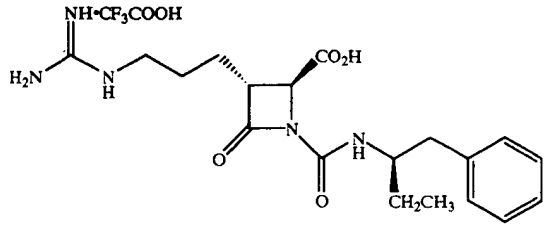
EXAMPLE 15



(homochiral)

Following the procedure of Example 1 but substituting methyl-(S)-(−)-2-isocyanato-3-phenylpropionate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 3(b), the desired product was obtained. IR (KBr) 1769 cm^{-1}, 1674 cm^{-1}, and 1632 cm^{-1}; MS $(M+H)^+$420.1.

EXAMPLE 16

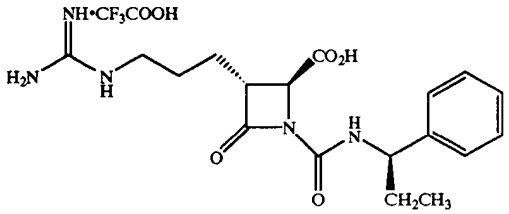


(homochiral)

a) R-(+)-1-Phenylpropylisocyanate

A solution of phosgene (20% in toluene, 5 ml) was diluted with toluene (10 ml). A mixture of R-(+)-1-phenylpropylamine (640 μl, 4.40 mmol), triethylamine (1.03 ml, 7.4 mmol) in toluene (5 ml) was added dropwise. Another 10 ml of toluene was added due to the difficulty of stirring. The reaction mixture was heated at reflux for 2 hours. TLC showed the completion of the reaction. The solvent was removed and the residue was stirred with ether (50 ml) for 10 minutes and filtered. The filtrate was concentrated to give the crude product which was purified by flash chromatography (silica, methylene chloride) to yield the desired product as a colorless oil (410 mg). IR 2262 cm^{-1}.

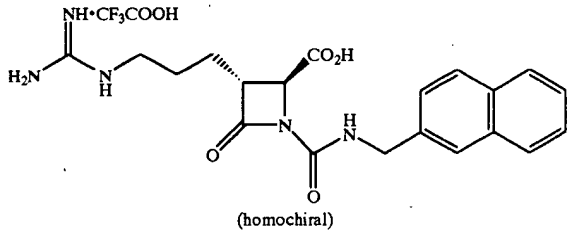
b)



(homochiral)

Following the procedure of Example 1 but substituting R-(+)-1-phenylpropylisocyanate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 1(e), the desired product was obtained. MS $(M+H)^+$ 376.1, $(2M+H)^+$ 751.2; IR (KBr) 1780 cm^{-1}, 1670 cm^{-}.

EXAMPLE 17



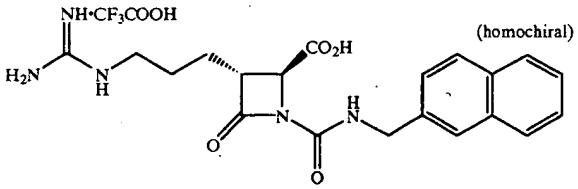
(homochiral)

a) 2-Naphthylmethylisocyanate

A drop of dimethylformamide and oxalyl chloride (1 ml) were added to a solution of 2-naphthylacetic acid (840 mg, 4.5 mmol) in methylene chloride (15 ml) at room temperature. This mixture was stirred at room temperature for 30 minutes. The solvent was removed and the residue was dissolved in acetone (15 ml) and cooled to 0° C. A solution of sodium azide (700 mg, 11 mmol) in water (10 ml) was added. The reaction mixture was stirred for 30 minutes at 5° C. and was then poured into a mixture of ice water (30 ml), ether (40 ml), and hexane (40 ml). The organic phase was

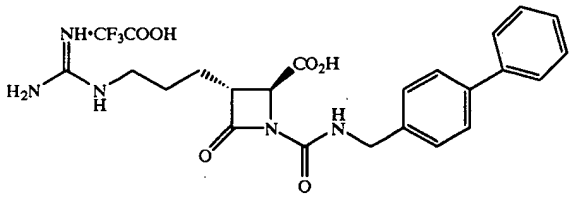
separated, washed with brine, dried and concentrated to 5 ml. Chloroform (5 ml) was added to the residue. The resultant solution was added to chloroform (10 ml) at 80° C. dropwise. The mixture was heated at reflux for 1 hour. The solvent was evaporated to give 680 mg of crude product. Purification by chromatography (silica, methylene chloride) gave 342 mg of the desired product as a white solid. IR (neat) 2355 cm^{-1}, 2336 cm^{-1}, 2267 cm^{-1}.

b)



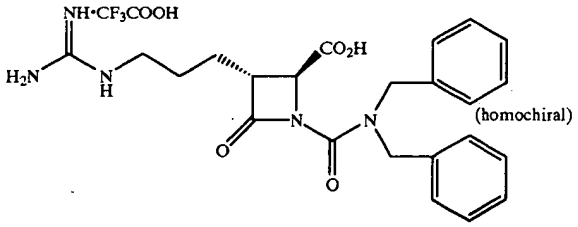
Following the procedure of Example 1 but substituting 2-naphthylmethylisocyanate for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 1(e), the desired product was obtained as a white fluffy powder. MS $(M+H)^+$ 398.1; IR (KBr) 1778 cm^{-1}, 1541 cm^{-1}.

EXAMPLE 18

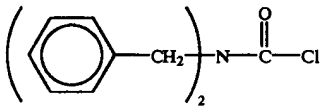


Following the procedure of Example 1, but substituting 4-isocyanatomethylbiphenyl for the benzyl isocyanate in step (d) followed by the deprotection and work-up described in Example 1(e), the desired product was obtained. IR (KBr) 1758 cm^{-1}; MS 424.1 $(M+H)^+$, 422.3 $(M-H)^-$.

EXAMPLE 19

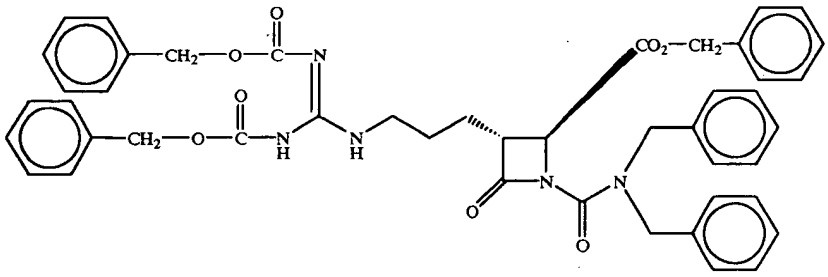


a)



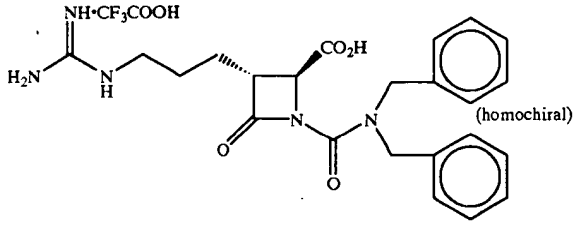
A solution of diphosgene (680 μl, 5.7 mmol) in toluene (5 ml) was added to a mixture of dibenzylamine (2.0 g, 9.8 mmol) and triethylamine (1.2 ml, 85 mmol) in toluene (15 ml). The resultant mixture was stirred at room temperature for 4 hours. The mixture was poured into 2N HCl aqueous solution (50 ml) and extracted with ethyl acetate (3×50 ml). The organic layers were combined, dried over magnesium sulfate and concentrated to give 2.60 g of the desired product as a white solid. IR (film) 1731 cm^{-1}.

b)



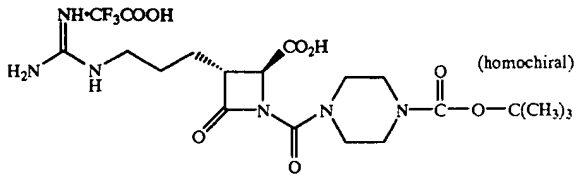
Triethylamine (42 μl), 4-dimethylaminopyridine (30 mg), and a solution of the carbamoyl chloride from step (a) (78 mg, 0.30 mmol) in methylene chloride (2 ml) were added to a solution of the benzyl ester product from Example 1(c) (116 mg, 0.20 mmol) in methylene chloride (2 ml). The mixture was stirred at room temperature for 3 days. Analytical HPLC indicated the reaction was complete. The reaction was quenched by the addition of 1N potassium bisulfate (15 ml). The mixture was extracted with ethyl acetate (100 ml). The organic layer was washed with brine (15 ml), dried over magnesium sulfate, and concentrated to give the crude product. Purification by flash chromatography (30% ethyl acetate/hexane) gave the desired product (95 mg). MS $(M+H)^+$ 796.1, $(M-H)^-$ 794.4; IR (film) 1785 cm^{-1}, 1732 cm^{-1}, 1671 cm^{-1}, 1639 cm^{-1}.

c)

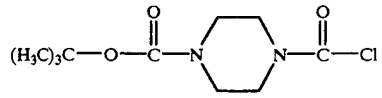


A mixture of the product from step (b) (95 mg, 0.12 mmol), 1N HCl (145 μl), and 10% palladium on carbon catalyst (61 mg) in dioxane (3 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 2 hours. Analytical HPLC indicated the reaction was complete. The reaction mixture was filtered through a Celite® cake and concentrated to give the crude product as a white powder. Purification by reverse phase HPLC using the solvent system described in Example 1(e) gives the desired product (36 mg) as a white powder. MS $(M+H)^+$ 438.1, $(M-H)^-$ 436.3; IR (KBr) 1786 cm^{-1}, 1672 cm^{-1}.

EXAMPLE 20

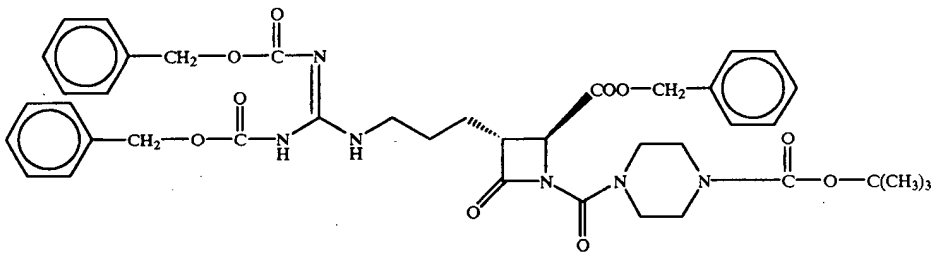


a)



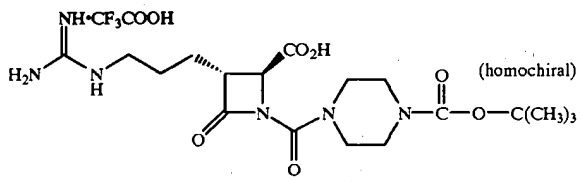
A mixture of tert-butylpiperizine carboxylate (1.0 g) and triethylamine (753 μl) in methylene chloride (5 ml) was added to a solution of diphosgene (326 μl, 20% in toluene) in methylene chloride at 0° C. The resultant mixture was stirred at 0° C. for 90 minutes. TLC showed completion of the reaction. The reaction was quenched by the addition of water (20 ml). The organic layer was separated. The aqueous layer was extracted with methylene chloride (2×20 ml. The organic layers were combined and washed with water (10 ml) and brine (2×10 ml), dried over magnesium sulfate, filtered and concentrated to give the crude product. Purification by flash chromatography (methylene chloride) provided 913 mg of the desired product as a white solid. IR (KBr) 1680 cm^{-1}, 1747 cm^{-1}.

b)



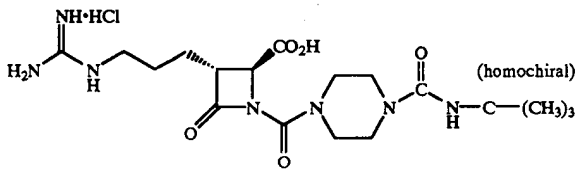
Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 180 μl, 0.18 mmol) was added dropwise to a −78° C. solution of the benzyl ester product of Example 1(c) (85 mg, 0.15 mmol) in tetrahydrofuran (3 ml). The mixture was stirred at −78° C. for 90 minutes. A solution of the chloro product from part (a) (45 mg, 0.18 mmol) in tetrahydrofuran (1 ml) was added. The reaction mixture was stirred at −78° C. for 5 hours. The reaction was quenched by the addition of 1N potassium bisulfate (15 ml). The mixture was extracted with ethyl acetate (3×30 ml). The organic layers were combined and washed with brine (2×15 ml), dried over magnesium sulfate, filtered and concentrated to give the crude product. Purification by flash chromatography (30–50% ethyl acetate/hexane) provided 32 mg of the desired product as a colorless oil. MS $(M+H)^+$ 785.4, $(M-H)^-$ 783.7; IR (neat) 1786 cm^{-1}, 1732 cm^{-1}, 1681 cm^{-1}, 1640 cm^{-1}.

c)

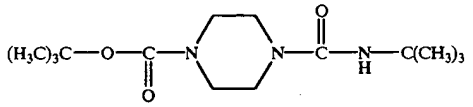


Deprotection and purification of the product from part (b) (32 mg) according to the procedure of Example 19(c) gives 17 mg of the desired product as a white fluffy powder. MS $(M+H)^+$ 427.1, $(M-H)^-$ 425.2; IR (KBr) 1792 cm^{-1}, 1670 cm^{-1}.

EXAMPLE 21

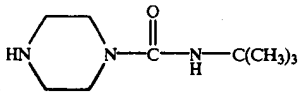


a)



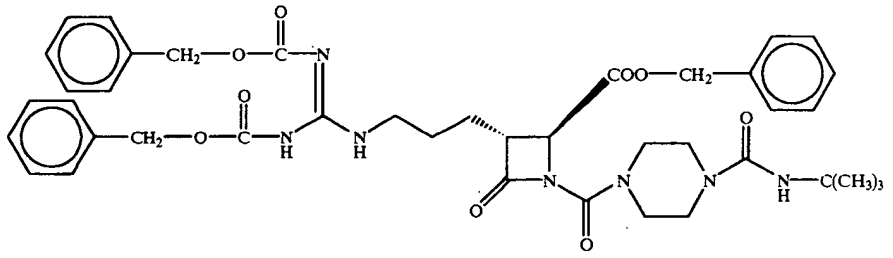
Tert-butyl isocyanate (0.28 g, 2.82 mmol) was added to a solution of a tert-butyl-1-piperazine carboxylate (0.5 g, 2.68 mmol) in methylene chloride (10 ml) and the mixture was stirred at room temperature. After 2 hours, the reaction mixture was evaporated in vacuo, suspended in water (50 ml) and extracted with ethyl acetate (2×50 ml). The organic phase was washed with saturated sodium chloride (1×50 ml) and filtered through a sintered glass funnel. The filtrate was dried over sodium sulfate and condensed to give 0.53 g of the desired product as a white solid.

b)



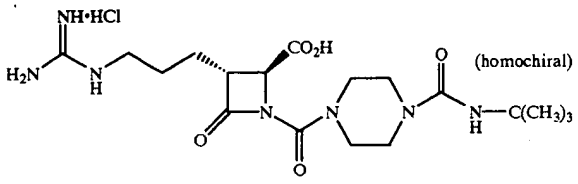
The product from part (a) (0.475 g, 1.67 mmol) was suspended in methylene chloride (4 ml) and trifluoroacetic acid (4 ml) was added over 1 minute. The mixture was stirred at room temperature. After 1 hour, the mixture was evaporated in vacuo. The residue was dissolved in water, the pH adjusted to 12–13 and extracted into ethyl acetate (2×25 ml). The organic phase was washed with saturated sodium chloride (1×50 ml), filtered, dried over sodium sulfate, and condensed to obtain 0.113 g of the desired product as a white solid.

c)



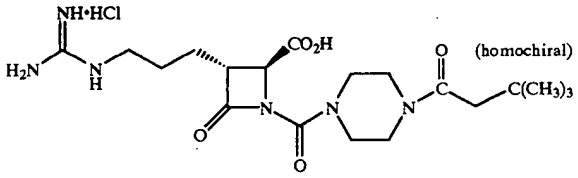
A solution of the benzyl ester product from Example 1(c) (65 mg, 0.114 mmol) in methylene chloride (1 ml) was cooled to −10° C. and triethylamine (24 μl, 0.17 mmol) was added, followed by the addition of 20% phosgene in toluene (0.15 ml, 0.285 mmol). After 90 minutes at −10° C., the mixture was evaporated in vacuo. The residue was taken up in methylene chloride (1 ml) and cooled to −10° C. Triethylamine (24 μl, 0.17 mmol) was added, followed by the addition of the piperazine product from part (b) (21 mg, 0.114 mmol). The mixture was stirred at −10° C. After 1 hour, the mixture was quenched with 10% monobasic potassium phosphate (15 ml) and extracted with ethyl acetate (2×20 ml). The organic phase was washed with saturated sodium chloride (1×30 ml), dried over sodium sulfate, and concentrated to obtain a pale yellow oil. Purification by preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid) gave 27 mg of the desired product as a white foam. MS 784.2 $(M+H)^+$; IR (film): 1787.1, 1741.9, 1636.6 cm^{-1}.

d)

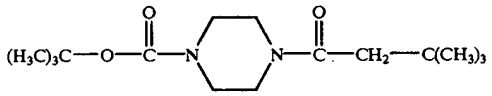


A solution of the product from part (c) (25 mg, 0.032 mmol) in 1,4-dioxane (7 ml) was treated with 1N HCl (40 μl, 0.038 mmol) and 10% palladium on carbon catalyst (15 mg). Hydrogen gas was bubbled through the mixture for 2 hours. The reaction mixture was filtered through a pad of Celite® which was then repeatedly washed with 1,4-dioxane. The combined eluents were lyophilized to give 15 mg of the desired product as a white lyophillate. MS 426.1 $(M+H)^+$, 424.3 $(M-H)^-$;IR (KBr) 1780 cm^-.

EXAMPLE 22

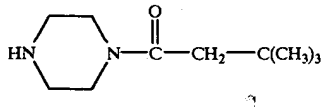


a)



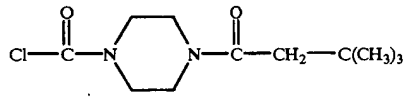
A solution of tert-butyl-1-piperazine carboxylate (0.5 g, 2.58 mmol) in methylene chloride (5 ml) was cooled to 0° C. N,N-Diisopropyl ethylamine (0.42 g, 3.22 mmol) and 4-dimethylaminopyridine (30 mg) were added, followed by the addition of tert-butyl acetyl chloride (0.36 g, 2.68 mmol) over 1 minute. The mixture was stirred at 0° C. for 2 hours. After two hours, the mixture was partitioned between water (20 ml) and ethyl acetate (2x20 ml). The organic layer was washed with brine (1x75 ml), dried over sodium sulfate and condensed to give 0.763 g of the desired product as a white solid. MS 285.0 $(M+H)^+$.

b)



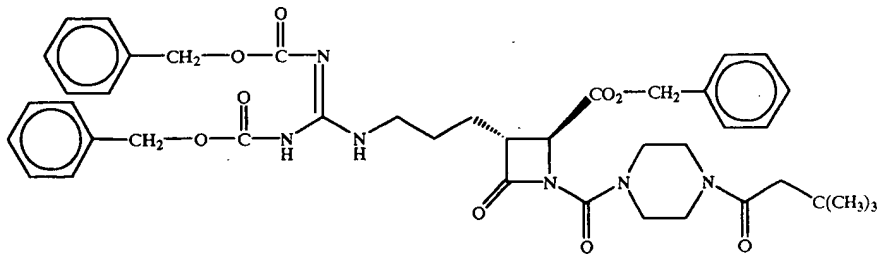
The product from part (a) (0.7 g, 2.46 mmol) was dissolved in methylene chloride (7 ml) and trifluoroacetic acid (4 ml) was added over 1 minute. The mixture was stirred at room temperature. After 1 hour, the mixture was evaporated in vacuo. The residue was dissolved in water, the pH was adjusted to 12–13 and extracted with ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate and condensed to give 0.218 g of the desired product as a pale yellow oil. MS 184.9 $(M+H)^+$.

c)



A solution of the product from part (b) (109 mg, 0.59 mmole) in methylene chloride (0.5 ml) was added to a mixture of phosgene (0.79 ml of 20% phosgene in toluene solution, 1.48 mmol) in methylene chloride (2 ml) at 0° C. followed by the addition of triethylamine (82 μl, 0.59 mmol). The mixture was stirred at 0° C. for 1 hour. The reaction mixture was then partitioned between water (25 ml) and ethyl acetate (2x25 ml). The organic phase was washed with 1N HCl (40 ml), brine (50 ml), dried over sodium sulfate and condensed to give a brown oil. Purification by flash chromatography (silica gel, 0–30% ethyl acetate/Hexane) gave 70 mg of the desired product.

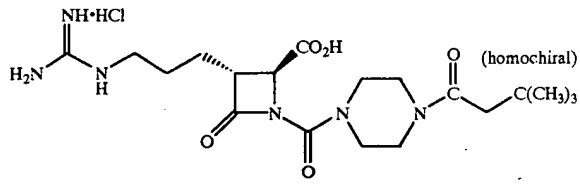
d)



The product from part (c) (70 mg, 0.122 mmol) was dissolved in tetrahydrofuran (1 ml) and cooled to -78° C. Sodium bis (trimethylsilyl)amide (0.15 ml, 0.146 mmol) was added over one minute and the mixture was stirred at -78° C. for 1 hour. A solution of the benzyl ester product from Example 1(c) (36 mg, 0.146 mmol) in tetrahydrofuran (0.5 ml) was added and the reaction mixture was stirred at

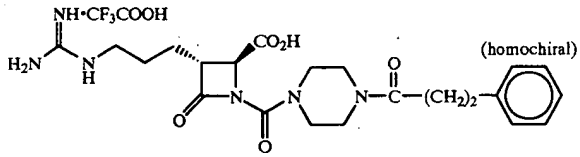
-78° C. After 2.5 hours, the reaction mixture was quenched with 0.5 N potassium bisulfate solution (25 ml) and extracted with ethyl acetate (2×25 ml). The organic phase was washed with brine (1×50 ml), dried over sodium sulfate and concentrated to a yellow oil. Purification by preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid) gave 21 mg of the desired product as a colorless oil. MS 783.4 $(M+H)^+$, 781.3 $(M-H)^-$.

e)

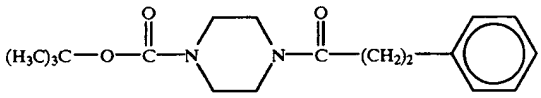


The product from part (d) was deprotected and worked-up as described in Example 21(d) to give 12 mg of the desired product as a white lyophillate. MS 425.1 $(M+H)^+$, 423.3 $(M-H)^-$; IR (KBr) 1786, 1736 cm^{-1}.

EXAMPLE 23

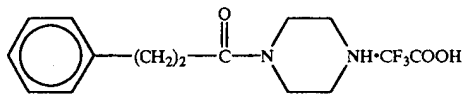


a)



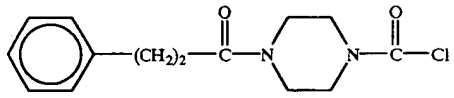
N,N-Diisopropylethylamine (560 μl), 4-dimethylaminopyridine (33 mg) and a solution of 3-phenylpropanoic acid chloride (400 μl, 2.69 mmol) in methylene chloride (2 ml) were added to a 0° C. solution of tert-butyl-1-piperazine carboxylate (500 mg, 2.68 mmol) in methylene chloride (4 ml). The mixture was stirred at 0° C. for 2 hours. The reaction was quenched with the addition of water (20 ml). The mixture was extracted with ethyl acetate (2×50 ml). The organic layers were combined and washed with brine (2×10 ml), dried over magnesium sulfate, and concentrated to give 872 mg of the desired product (crude) as a yellow solid. MS $(M+H)^+$ 319.1.

b)



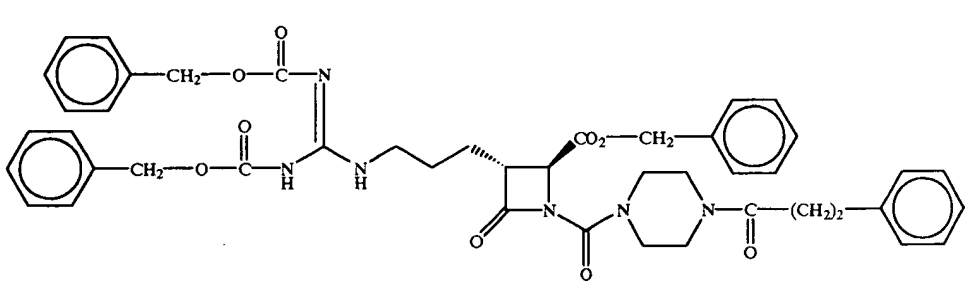
A mixture of the crude product from part (a) (860 mg, 2.68 mmol), trifluoroacetic acid (10 ml) and methylene chloride (10 ml) was stirred at room temperature for 1 hour. TLC showed the reaction was complete. The solvent was removed and 1N sodium hydroxide solution (15 ml) was added. The mixture was extracted with ethyl acetate (100 ml). The combined organic solution was washed with brine (10 ml), dried over magnesium sulfate, filtered and concentrated to give 238 mg of the desired product as a yellow oil which was used without further purification. IR(film) 1633 cm^{-1}.

c)



A mixture of the product from part (b) (200 mg, 0.92 mmol) and triethylamine (154 μl) in methylene chloride (4 ml) was added to a solution of phosgene in toluene (584 g, 20%) at 0° C. The resulting mixture was stirred at 0° C. for 20 minutes. TLC showed the completion of the reaction. The solvent was removed, and anhydrous ether (50 ml) was added. The mixture was filtered and the filtrate was concentrated to give the crude product as a yellow oil. Purification of the crude product by flash chromatography (50% ethyl acetate/hexanes) provided 235 mg of the desired product as a yellow oil. IR(film) 1741 cm^{-1}, 1703 cm^{-1}.

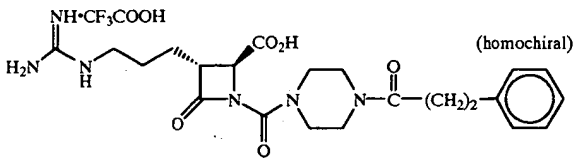
d)



Sodium bis(trimethylsilyl)azide (1.0M in tetrahydrofuran, 210 μl, 0.21 mmol) was added dropwise to a -78° C. solution of the benzyl ester product from Example 1(c) (100 mg, 0.17 mmol) in tetrahydrofuran (2 ml). The mixture was stirred at -78° C. for 1 hour. A solution of the product from part (c) (58 mg, 0.21 mmol) in tetrahydrofuran (1 ml) was added. The reaction mixture was stirred at -78° C. for 2.5 hours. Analytical HPLC indicated that the starting material

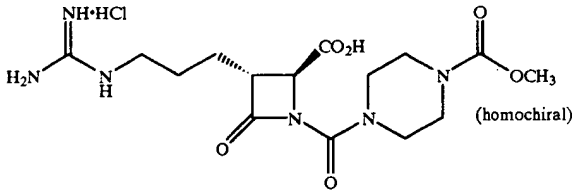
was not completely consumed. This was quenched with the addition of 1N potassium bisulfate (20 ml). The mixture was extracted with ethyl acetate (2×30 ml). The organic layers were combined and washed with brine (2×15 ml) dried (magnesium sulfate), filtered and concentrated to give the crude product. Purification of the crude product by reverse phase HPLC provided 32 mg of the desired product as a colorless oil. MS $(M+H)^+$ 817.1, $(M-H)^-$ 815.4.

e)

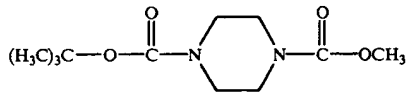


Deprotection and purification of the product from part (d) according to the procedure of Example 19(c) gave 10 mg of the desired product as a white powder. MS $(M+H)^+$ 459.2, $(M-H)^-$ 457.4; IR (KBr) 1790 cm^{-1}, 1680 cm^{-}.

EXAMPLE 24

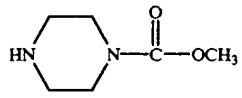


a)



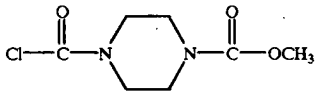
A solution of tert-butyl-1-piperazine carboxylate (0.5 g, 2.68 mmol) in methylene chloride (5 ml) was cooled to 0° C. N,N-Diisopropylethylamine (0.42 g, 3.22 mmol) and 4-dimethylaminopyridine (30 mg) were added, followed by addition of methyl chloroformate (0.25 g, 2.69 mmol) over 1 minute. The mixture was stirred at 0° C. for 1 hour. The mixture was partitioned between water (20 ml) and ethyl acetate (2×20 ml). The organic phase was washed with brine (1×75 ml), dried over sodium sulfate and condensed to give the desired product as a cream colored solid (0.636 g).

b)



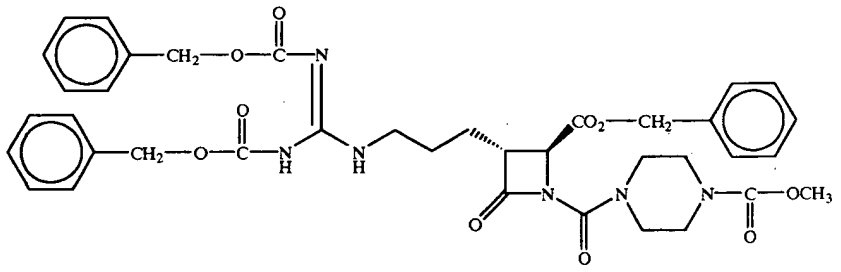
The product from part (a) (0.3 g, 1.23 mmol) was dissolved in methylene chloride (3 ml) and cooled to 0° C. Trifluoroacetic acid (3 ml) was added and the mixture was warmed to room temperature and stirred for 1 hour. The mixture was then evaporated in vacuo. The residue was dissolved in water, the pH adjusted to 12–13 with 6 N sodium hydroxide, and extracted with ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate and condensed to give 91 mg of the desired free amine product. IR(film) 1696.2 cm^{-}; MS 144.9 $(M+H)^+$.

c)



A mixture of the product from part (b) (89 mg, 0.617 mmol) and triethylamine (86 μl, 0.59 mmol) in methylene chloride (2 ml) was added to a mixture of phosgene (0.82 ml of a 20% phosgene in toluene solution, 1.54 mmol). The mixture was stirred at 0° C. for 1 hour. The reaction mixture was then partitioned between water (25 ml) and ethyl acetate (2×25 ml). The organic phase was washed with 1N HCl (40 ml), brine (50 ml), dried over sodium sulfate and concentrated to give 108 mg of the desired product as a brown oil. IR (film) 1738.8, 1704.7 cm^{-1}.

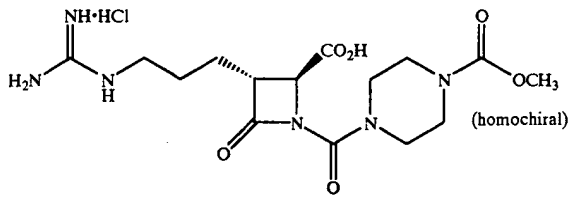
d)



The benzyl ester product from Example 1(c) (111 mg, 0.194 mmol) was dissolved in tetrahydrofuran (2 ml) and cooled to −78° C. Sodium bis(trimethylsilyl)amide (0.23 ml, 0.234 mmol) was added over 1 minute and the mixture was stirred at −78° C. for 1 hour. A solution of the product from part (c) (48 mg, 0.234 mmol) in tetrahydrofuran (1 ml) was added and the reaction mixture was stirred at −78° C. After

1 hour, the mixture was quenched with 0.5 N potassium bisulfate solution (25 ml) and extracted with ethyl acetate (2×25 ml). The organic phase was washed with brine (1×50 ml), dried over sodium sulfate and concentrated to give a pale yellow oil. Purification by preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid) gave 25 mg of the desired product as a colorless oil/foam. MS 743.1 $(M+H)^+$, 741.4 $(M-H)^-$; IR(film) 1786.6 cm^{-1}.

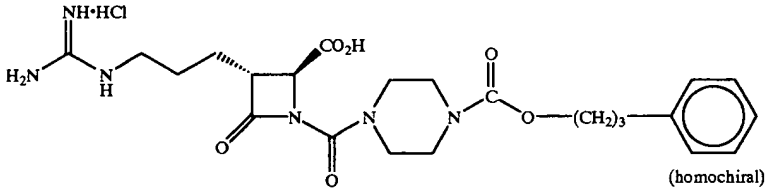
e)



(homochiral)

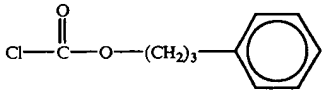
Deprotection of the product from part (d) (22 mg, 0.027 mmol) and work-up as described in Example 21(d) gave 12 mg of the desired product as a white lyophilate. MS 385.1 $(M+H)^+$, 383.2 $(M-H)^-$; IR(film) 1786 cm^{-1}.

EXAMPLE 25



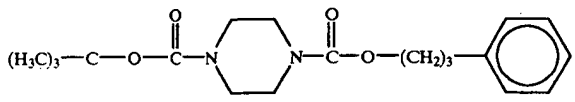
(homochiral)

a)



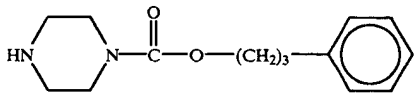
3-Phenyl-1-propanol (1.09 g, 7.34 mmol) was added to a mixture of phosgene (5.5 ml of 20% phosgene in toluene solution, 11.01 mmol) in methylene chloride (5 ml) at 0° C. The mixture was stirred at 0° C. for 5 hours. The reaction mixture was evaporated in vacuo to give a colorless oil. Purification by flash column chromatography (silica gel, 0–5% ethyl acetate/Hexane gave 1.33 g of the desired product.

b)



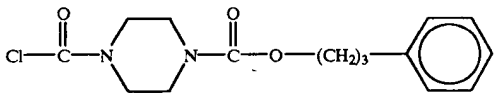
A solution of tert-butyl-1-piperazine carboxylate (0.2, 1.07 mmol) in methylene chloride (3 ml) was cooled to 0° C. N,N-Diisopropylethylamine (0.25 g, 1.93 mmol) and 4-dimethylaminopyridine (5–7 crystals) were added, followed by addition of a solution of the product from part (a) (0.2 g, 1.07 mmol) over 1 minute. The mixture was stirred at 0° C. for 1 hour. The mixture was then partitioned between water (20 ml) and ethyl acetate (2×20 ml). The organic layer was washed with brine (1×75 ml), dried over sodium sulfate and concentrated to give 0.35 g of the desired product as a yellow oil.

c)



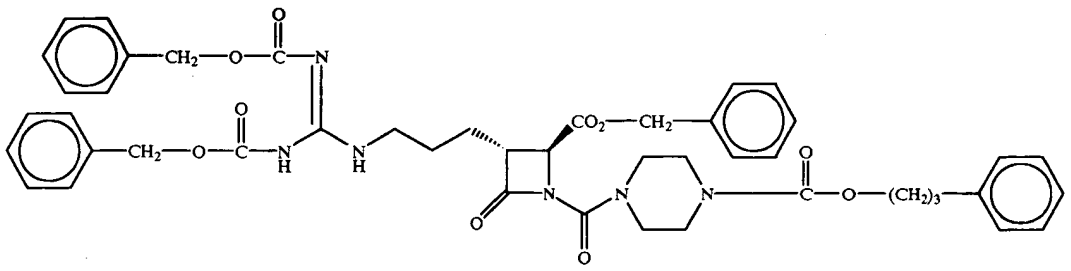
The product from part (b) (0.35 g, 1.03 mmol) was dissolved in methylene chloride (4 ml) and cooled to 0° C. Trifluoroacetic acid (4 ml) was added over 1 minute and the mixture was warmed to room temperature and stirred for 1 hour. The mixture was then evaporated in vacuo. The residue was dissolved in water, the pH was adjusted to 12–13 using 6 N sodium hydroxide and extracted with ethyl acetate. The organic phase was washed with brine, filtered over sodium sulfate and concentrated to give 0.23 g of the desired product as a white solid. MS 248.9 (M +H)

d)



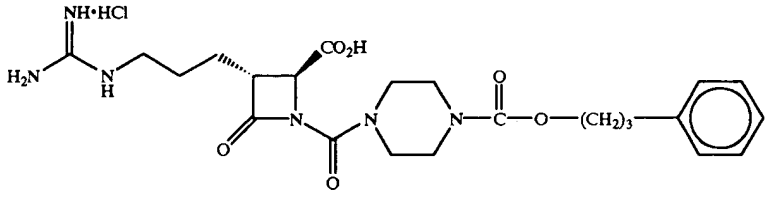
A solution of the product from part (c) (100 mg, 0.403 mmol) in methylene chloride (1 ml) was added to a mixture of phosgene (0.53 ml of a 20% phosgene in toluene solution, 1.01 mmol) followed by the addition of triethylamine (60 μl, 0.403 mmol). The mixture was stirred at 0° C. for 1 hour. The reaction mixture was then partitioned between water (25 ml) and ethyl acetate (2×25 ml). The organic phase was washed with 1N HCl (40 ml), brine (50 ml), dried over sodium sulfate and concentrated to give 115 mg of the desired product.

e)



A solution of the benzyl ester product from Example 1(c) (43 mg, 0.075 mmol) in methylene chloride (1 ml) was cooled to 0° C. and triethylamine (11 mg, 0.113 mmol) and 4-dimethylaminopyridine (6–8 crystals) were added. A solution of the product from part (d) (58 mg) in methylene chloride (0.5 ml) was added and the mixture was stirred at 0° C. for 45 minutes followed by stirring at room temperature for 3 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash column chromatography (silica gel, 30% ethyl acetate/hexane) gave 40 mg of the desired product as a pale yellow oil. MS 847.1 $(M+H)^+$, 845.4 $(M-H)^-$; IR (film) 1784 cm^-.

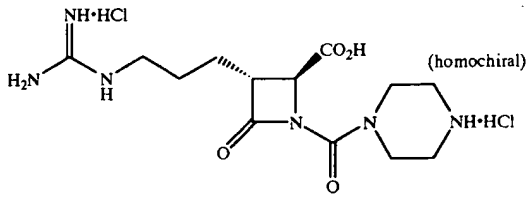
f)



(homochiral)

Deprotection of the product from part (e) (40 mg, 0.027 mmol) and work-up as described in Example 21(d) gave 21 mg of the desired product as a white lyophilate. MS 489.1 $(M+H)^+$, 487.4 $(M-H)^-$; IR (KBr) 1784, 1667 cm^{-1}.

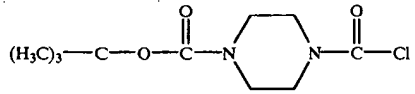
EXAMPLE 26



(homochiral)

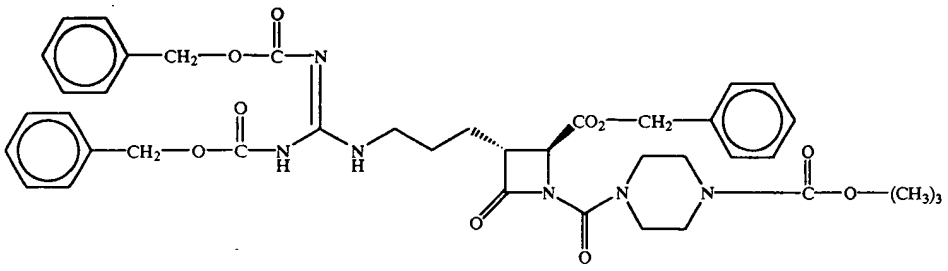
a)

-continued



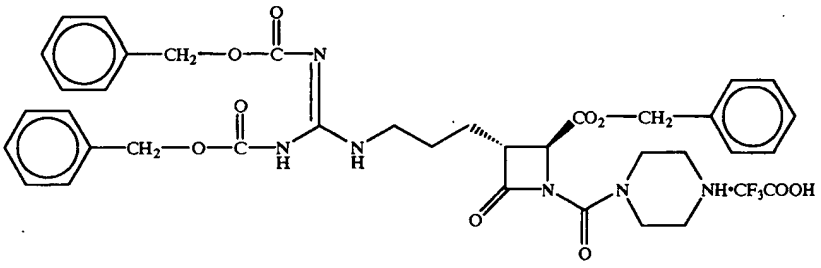
A solution of tert-butyl-1-piperazine carboxylate (0.5 g, 2.8 mmol) in methylene chloride (1 ml) was added to a mixture of phosgene (3.6 ml of 20% phosgene in toluene solution, 6.71 mmol) in methylene chloride (2 ml) at 0° C. Triethylamine (0.27 g, 6.71 mmol) was then added and the mixture was stirred at 0° C. for 1 hour. The mixture was then partitioned between water (30 ml) and ethyl acetate (2×30 ml). The organic layer was washed with brine (1×60 ml), dried over sodium sulfate and condensed to give crude product. Purification of the crude product by flash chromatography (silica gel, 40% ethyl acetate/hexane) gave 0.515 g of the desired product as a white solid. IR (film) 1737.6, 1697.0 cm^{-1}.

b)



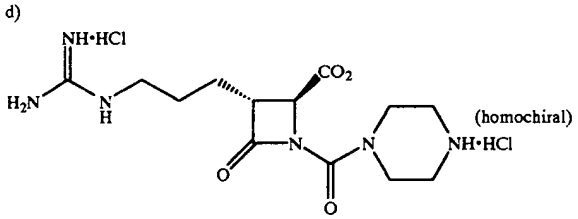
A solution of the benzyl ester product from Example 1(c) (47 mg, 0.082 mmol) in methylene chloride (1 ml) was cooled to 0° C. and triethylamine (12 mg, 0.123 mmol) and 4-dimethylaminopyridine (6–8 crystals) were added. A solution of the product from part (a) (51 mg) in methylene chloride (1 ml) was added and the mixture was stirred at 0° C. for 40 minutes followed by stirring at room temperature for 4–5 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash chromatography (silica gel, 0–30% ethyl acetate/hexane) gave 48 mg of the desired product as a colorless oil. MS 785.1 $(M+H)^+$, 783.4 $(M-H)^-$.

c)



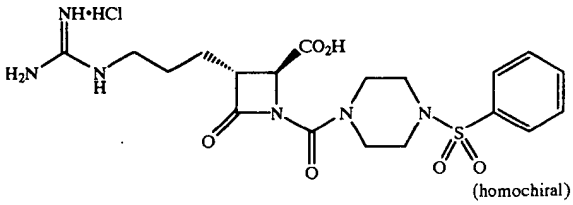
The product from part (b) (40 mg, 0.051 mmol) was dissolved in methylene chloride (1 ml) and cooled to 0° C. Trifluoroacetic acid (1 ml) was added over 1 minute and the mixture was warmed to room temperature and stirred for 1 hour. The mixture was then evaporated in vacuo to give the desired product as a yellow oil which was used in the next step without further purification. MS 685.1 $(M+H)^+$, 683.3 $(M-H)^-$.

d)

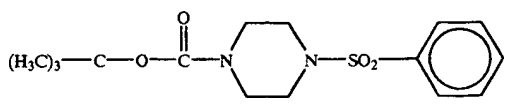


The product from part (c) (49 mg, 0.061 mmol) was deprotected and worked-up as described in Example 21(d) to give 15 mg of the desired product as a white lyophilate. MS 327.0 $(M+H)^+$, 325.0 $(M-H)^-$; IR (KBr) 1786, 1653 cm^{-1}.

EXAMPLE 27



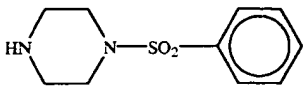
a)



A solution of tert-butyl-1-piperazine carboxylate (0.2 g, 1.07 mmol) in methylene chloride (2 ml) was cooled to 0° C. N,N-diisopropylethylamine (0.167 g, 1.28 mmol) and 4-dimethylaminopyride (30 mg) were added, followed by addition of benzenesulfonyl chloride (0.19 g, 1.07 mmol) over 1 minute. The mixture was stirred at 0° C. for 2 hours. After two hours, water (20 ml) was added to the mixture and extracted with ethyl acetate (2×20 ml). The organic layer

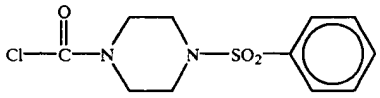
was washed with brine (1×75 ml), dried over sodium sulfate and concentrated to give 0.35 g of the desired product as a white solid.

b)



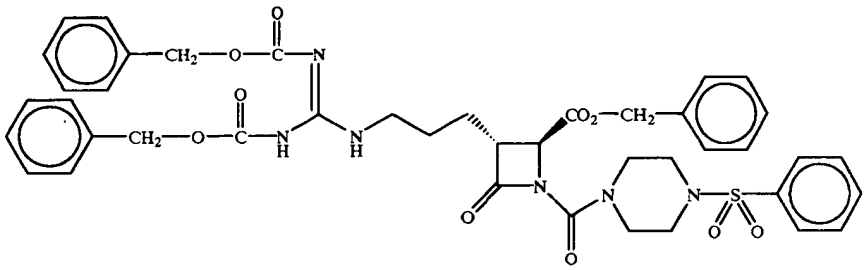
The product from part (a) (0.35 g, 1.07 mmol) was dissolved in methylene chloride (3 ml) and cooled to 0° C. Trifluoroacetic acid (3 ml) was added over 1 minute and the mixture was warmed to room temperature and stirred for 1 hour. The mixture was then evaporated in vacuo. The residue was dissolved in water, the pH adjusted to 12–13 using 6 N sodium hydroxide and extracted with ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate and concentrated to give 0.208 g of the desired product as a pale yellow oil. MS 226.8 $(M+H)^+$.

c)



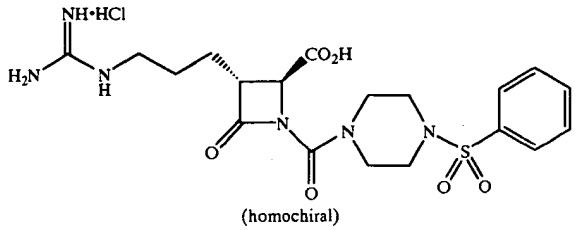
A mixture of the product from part (b) (100 mg, 0.442 mmol) and triethylamine (62 μl, 0.442 mmol) in methylene chloride (1 ml) was added to a mixture of phosgene (0.59 ml of a 20% phosgene in toluene solution, 1.1 mmol). The mixture was stirred at 0° C. for 1 hour. The mixture was then evaporated in vacuo. The residue was suspended in ether and filtered. The eluents were concentrated to give 100 mg of the desired product as a cream colored solid.

d)



A solution of the benzyl ester product from Example 1(c) (64 mg, 0.112 mmol) in methylene chloride (1 ml) was cooled to 0° C. and triethylamine (17 mg, 0.168 mmol) and 4-dimethylaminopyridine (6–8 crystals) were added. The product from part (c) (58 mg, 0.168 mmol) was added and the mixture was stirred at 0° C. for 45 minutes followed by stirring at room temperature for 3 hours. The mixture was then evaporated in vacuo to give crude product. Purification of the crude product by preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid) gave 28 mg of the desired product as a colorless oil. MS 825.1 $(M+H)^+$.

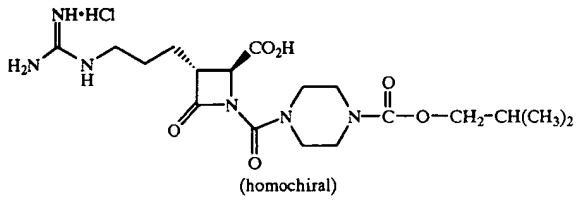
e)



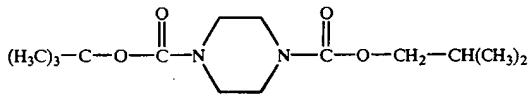
(homochiral)

The product from part (d) (25 mg, 0.03 mmol) was deprotected and worked-up as described in Example 21(d) to give 12 mg of the desired product as a white lyophilate. MS 467.0 $(M+H)^+$, 465.3 $(M-H)^-$;IR (film) 1787.25, 1662.13 cm^{-1}.

EXAMPLE 28

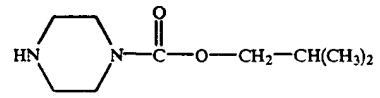


a)



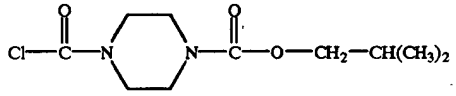
Diisopropylethylamine (30 mg), 4-dimethylaminopyridine (30 mg) and a solution of isobutyl chloroformate (366 μl, 268 mmol) in methylene chloride (2 ml) were added to a 0° C. solution of tert-butyl-1-piperazine carboxylate (500 mg, 2.68 mmol) in methylene chloride (2 ml). The mixture was stirred at 0° C. for 1 hour. The reaction was quenched with the addition of water (20 ml). The mixture was extracted with ethyl acetate (2×50 ml). The organic layers were combined and washed with brine (2×10 ml), dried over magnesium sulfate, and concentrated to give 819 mg of the desired product as a yellow solid; IR(film) 1701 cm^{-1}, 1688 cm^{-1}.

b)



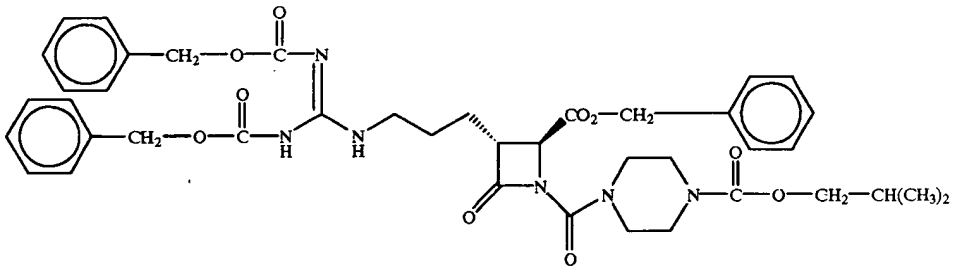
A mixture of the crude product from part (a) (800 mg, 2.79) mmol), trifluoroacetic acid (10 ml) and methylene chloride (10 ml) was stirred at room temperature for 1 hour. TLC showed the completion of the reaction. The solvent was removed and 1N sodium hydroxide solution (15 ml) was added. The mixture was extracted with ethyl acetate (100 ml). The combined organic solution was washed with brine (10 ml), dried over magnesium sulfate, filtered and concentrated to give 511 mg of the desired product as a yellow oil which was used without further purification. IR(film) 1692 cm^{-1}.

c)



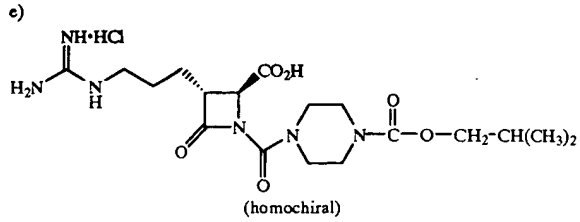
A mixture of the product from part (b) (477 mg, 2.56 mmol) and triethylamine (432 μL) in methylene chloride (5 ml) was added to a solution of phosgene in toluene (1.6 ml, 20%) at 0° C. The resultant mixture was stirred at 0° C. for 2 hours. TLC showed the completion of the reaction. The solvent was removed, and anhydrous ether (50 ml) was added. The mixture was filtered and the filtrate was concentrated to give the crude product (481 mg) as an orange oil. Purification of the crude product provided 453 mg of the desired product as a yellow oil. IR (film) 1741 cm^{-1}, 1703 cm^{-1}.

d)



Triethylamine (36 μl), 4-dimethylaminopyridine (30 mg) and a solution of the product from step (c) (65 mg, 0.24) in methylene chloride (1 ml) were added to a solution of the benzyl ester product from Example 1(c) (100 mg, 0.17 mmol) in methylene chloride (1 ml). The mixture was stirred for 4 hours at room temperature. Analytical HPLC indicated that the reaction was complete. The reaction was quenched by the addition of 1N potassium bisulfate (15 ml). The mixture was extracted with ethyl acetate (100 ml). The organic layer was washed with brine (15 ml), dried over magnesium sulfate, and concentrated to give the crude product as a yellow oil. Purification by flash chromatography (50% ethyl acetate/hexane) gave 81 mg of the desired product. MS 785.2 $(M+H)^+$, 783.4 $(M-H)^-$.

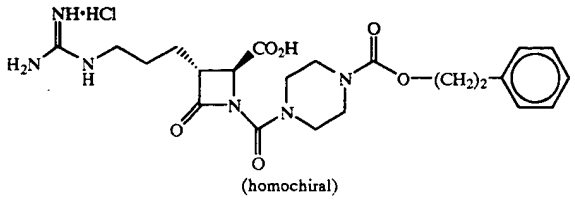
c)



(homochiral)

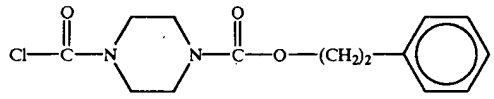
The product from part (d) (80 mg, 0.10 mmol) was deprotected and worked-up as described in Example 21(d) to give 41 mg of the desired product as a white solid. MS $(M+H)^+$ 427.1, $(M-H)^-$ 425.3; IR (KBr) 1786 cm^{-1}, 1653 cm^{-1}.

EXAMPLE 29



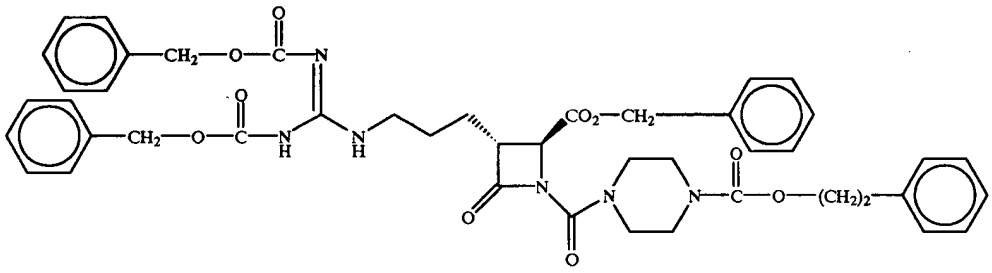
(homochiral)

a)



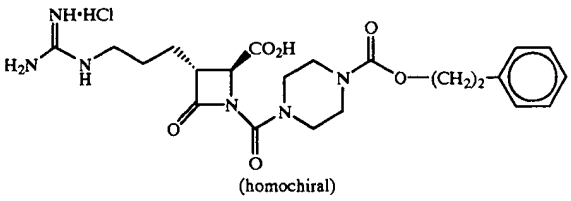
Following the procedure of Example 25(a) through (d) but substituting phenethyl alcohol for the 3-phenyl-1-propanol in (a), the desired product was obtained as an orange oil.

b)



A solution of the benzyl ester product from Example 1(c) (69 mg, 0.121 mmol) in methylene chloride (1 ml) was cooled to 0° C. and triethylamine (18 mg, 0.181 mmol) and 4-dimethylaminopyridine (6–8 crystals) were added. A solution of the product from part (a) (54 mg) in methylene chloride (1 ml) was added and the mixture was stirred at 0° C. for 45 minutes followed by stirring at room temperature for 2.5 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash chromatography (silica gel, 0–30% ethyl acetate/Hexane) gave 90 mg of the desired product as a colorless oil. MS 833.1 $(M+H)^+$, 831.4 $(M-H)^-$; IR (film) 1786, 1737.7 cm^{-1}.

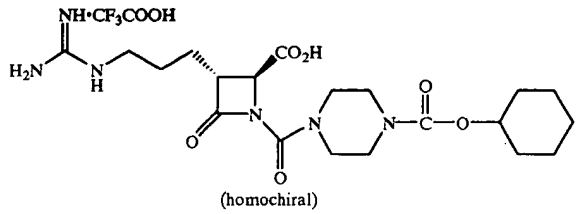
c)



(homochiral)

The product from part (b) (85 mg, 0.108 mmol) was deprotected and worked-up as described in Example 21(d) to give 34 mg of the desired product as a white lyophilate. MS 475.1 $(M+H)^+$, 473.4 $(M-H)^-$; IR (film) 1783 cm^{-1}, 1665 cm^{-1}.

EXAMPLE 30

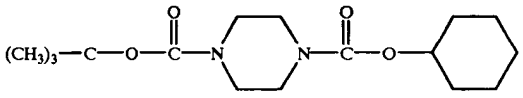


(homochiral)

a) Cyclohexyl chloroformate

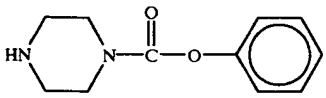
A mixture of cyclohexanol (500 mg, 5.0 mmol) and triethylamine (836 μl) in methylene chloride (4 ml) was added to a 0° C. solution of phosgene in toluene (5.3 ml, 20%). The resultant mixture was stirred at 0° C. for 2.5 hours. TLC showed completion of the reaction. The solvent was removed and anhydrous ether (50 m l) was added. The mixture was filtered and the filtrate was concentrated to give 726 mg of the desired product as a colorless oil which was used without further purification. IR (film) 1776 cm^{-1}.

b)



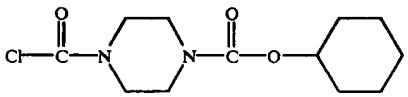
Disopropylethylamine (560 μl), 4-dimethylaminopyridine (30 mg) and a solution of cyclohexyl chloroformate (473 mg, 2.68 mmol) in methylene chloride (2 ml) were added to a 0° C. solution of tert-butyl-1-piperazine carboxylate (500 mg, 2.68 mmol) in methylene chloride (3 ml). The mixture was stirred at 0° C. and warmed to room temperature over 4 hours. The reaction was quenched with the addition of water (15 ml). The mixture was extracted with ethyl acetate (100 ml). The organic layer was washed with brine (2x10 ml), dried over magnesium sulfate, and concentrated to give 832 mg of the desired product as a white solid. IR(film) 1692 cm^{-1}.

c)



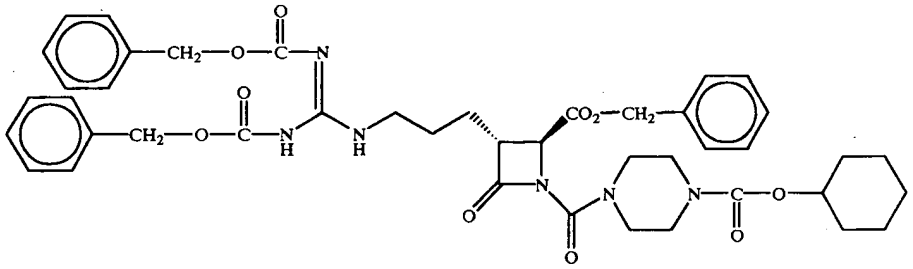
A mixture of the crude product from part (b) (832 mg, 2.68 mmol), trifluoroacetic acid (2 ml) and methylene chloride (2 ml) was stirred at room temperature for 3 hours. TLC showed completion of the reaction. The solvent was removed and 1N sodium hydroxide solution (15 ml) was added. The mixture was extracted with ethyl acetate (100 ml). The combined organic solution was washed with brine (10 ml), dried over magnesium sulfate, filtered and concentrated to give 535 mg of the desired product as a light yellow oil which was used without further purification.

d)



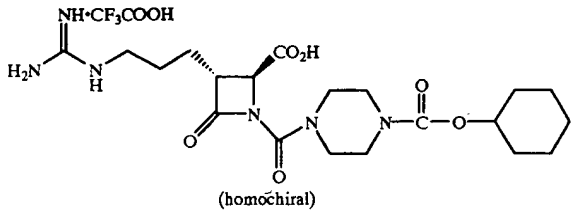
A mixture of the product from part (c) (459 mg) and triethylamine (448 μl) in methylene chloride (2 ml) was added to a solution of phosgene in toluene (2.1 ml, 20%) at 0° C. The resultant mixture was stirred at 0° C. for 1 hour. TLC showed the completion of the reaction. The solvent was removed and anhydrous ether (50 ml) was added. The mixture was filtered and the filtrate was concentrated to give the crude product (626 mg) as an orange oil. Purification of the crude product provided 626 mg of the desired product as a yellow solid. IR (film) 1740 cm^{-1} 1697 cm^{-1}.

e)



Triethylamine (34 μl), 4-dimethylaminopyridine (20 mg) and a solution of the product from part (d) (65 mg, 0.24 mmol) in methylene chloride (1 ml) was added to solution of the benzyl ester product from Example 1(c) (113 mg, 0.14 mmol) in methylene chloride (1 ml). The mixture was stirred at room temperature for 2 hours. Analytical HPLC showed the reaction was complete. The reaction was quenched with the addition of 1N potassium bisulfate (15 ml). The mixture was extracted with ethyl acetate (100 ml). The organic layer was washed with brine (15 ml), dried (magnesium sulfate) and concentrated to give the crude product as a colorless oil. Purification by flash chromatography (50% ethyl acetate/hexane) gave 113 mg of the desired product. IR (film) 1786 cm^{-1}, 1734 cm^{-1}, 1683 cm^{-1}, 1639 cm^{-1}.

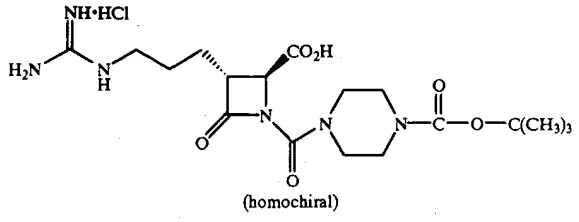
f)



(homochiral)

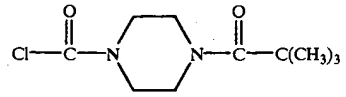
Deprotection and purification of the product from part (e) (113 mg, 0.14 mmol) according to the procedure of Example 19(c) gives 33 mg of the desired product as a white solid. MS $(M+H)^+$ 453.3, $(M-H)^-$ 451.5; IR (KBr) 1790 cm^{-1}, 1674 cm^{-1}.

EXAMPLE 31



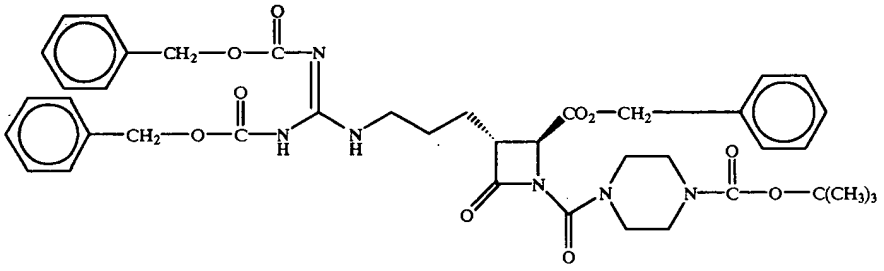
(homochiral)

a)



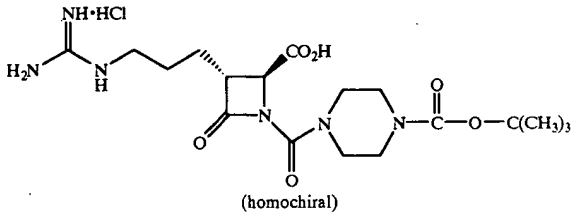
Following the procedure of Example 22(a) through (c) but substituting tert-butylcarbonyl chloride for the tert-butyl acetylchloride in part (a), the desired product was obtained as a pale brown solid. IR (film) 1733.2, 1616.4 cm^{-1}.

b)



Triethylamine (24 mg, 0.236 mmol) and 4-dimethylaminopyridine (8–10 crystals) were added to a solution of the benzyl ester product from Example 1(c) (90 mg, 0.157 mmol) in methylene chloride (1 ml). A solution of the product from part (a) (59 mg, 0.236 mmol) in methylene chloride (1 ml) was added and the mixture was stirred at 0° C. for 30 minutes followed by stirring at room temperature for 6 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash chromatography (silica gel, 0–30% ethyl acetate/Hexane) gave 89 mg of the desired product as a colorless. MS 769.4 $(M+H)^+$, 767.6 $(M-H)^-$; IR (film) 1785.4, 1733.4, 1679.4, 1635.9 cm^{-1}.

c)



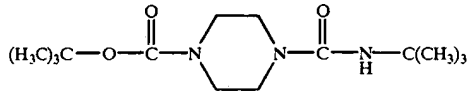
(homochiral)

The product from part (b) (87 mg, 0.11 mmol) was deprotected and worked-up as described in Example 21(d) to give 10 mg of the desired product as a white solid lyophilate. MS 411.2 $(M+H)^+$, 409.5 $(M-H)^-$; IR (KBr) 1788.0, 1742.0 cm^{-1}. QA206b

EXAMPLE 32

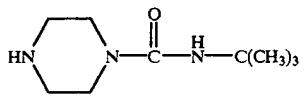
The product of Example 21 was also prepared as follows:

a)



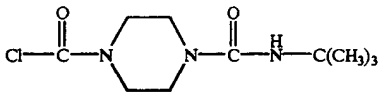
A solution of tert-butylisocyanate (10.28 g, 103 mmol) in methylene chloride (20 ml) was added over 2 minutes to a solution of tert-butyl-1-piperazine carboxylate (9.17 g, 49.2 mmol) in methylene chloride (40 ml) at 0° C. under nitrogen. After stirring the reaction mixture at room temperature for 2 hours, the reaction mixture was poured into hexane (60 ml). The resulting precipitate was collected by filtration and washed with hexane/methylene chloride (2:1) (2×50 ml). The combined eluent was concentrated to approximately a 20 ml volume and the precipitate that formed was collected by filtration, washed as above and combined with previously collected solid. The solid was dried under vacuum to give 14.1 g of the desired product. MS 286.2 $(M+H)^+$.

b)



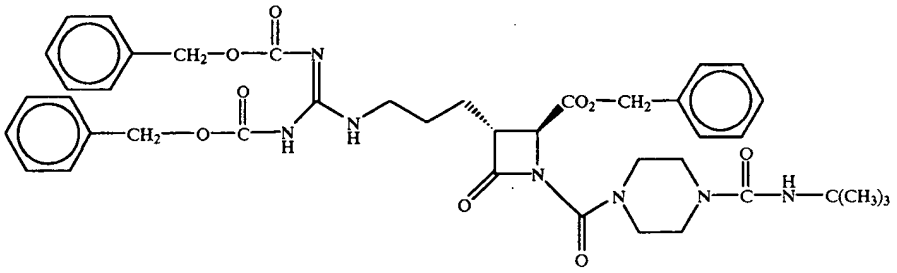
Trifluoroacetic acid (25 ml) was added dropwise over 5 minutes to a solution of the product from part (a) (14.1 g, 49 mmol) in methylene chloride (25 ml) at 0° C. under nitrogen. The reaction mixture was stirred at 0° C. for 20 minutes and then at room temperature for 2 hours. The reaction mixture was transferred to a beaker (100 ml) and diluted with ethyl acetate (200 ml) and water (200 ml). While vigorously stirring the biphasic mixture sodium hydroxide (25% aqueous) was added dropwise until the pH of the aqueous phase was about 12. The organic phase was separated with an additional portion of ethyl acetate (200 ml). The combined organics were dried over sodium sulfate, filtered and concentrated to give 11 g of the desired product as a white solid.

c)



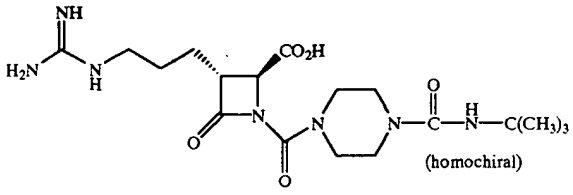
A solution of the product from part (b) (about 11 g) in methylene chloride: acetonitrile (1:1, 40 ml) was added dropwise over 10 minutes to a solution of phosgene (20% in toluene, 70 ml, 132 mmol) at 0° C. under nitrogen. Triethylamine (30 ml) was then added dropwise over 5 minutes. The reaction mixture was then stirred at room temperature for 2 hours. The reaction mixture was transferred to a separatory funnel, diluted with ethyl acetate (150 ml) and washed with 2N HCl (2×150 ml). The organics were dried over sodium sulfate, filtered and concentrated to a yellow oil. Purification by flash chromatrography (silica gel, 0 to 50% ethyl acetate in hexane) provided 7.8 g of the desired product.

d)



The carbamoyl chloride product from part (c) (4.5 g, 18.2 mmol), triethylamine (2.6 ml, 18.2 mmol) and dimethylaminopyridine (225 mg) were added to a solution of the benzyl ester product from Example 1(c) (6.94 g, 12.13 mmol) in methylene chloride (50 ml) at room temperature under nitrogen. After stirring the reaction mixture at room temperature for 4 hours, additional portions of the acid chloride product from part (c) (1 g, 4 mmol) and triethylamine (1 ml, 7 mmol) were added. The reaction was stirred for an additional 3 hours. The reaction was diluted with hexane (5 ml) and the crude reaction mixture was loaded onto a silica column (wetted with hexane) for purification by flash chromatography (0 to 60% ethyl acetate in hexane) to provide 7.46 g of the desired product. MS 784.4 $(M+H)^+$, 782.2 $(M-H)^-$.

e)

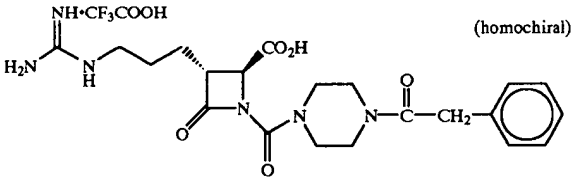


(homochiral)

Water (50 ml), concentrated HCl (0.8 ml, 9.6 mmol) and 10% palladium on carbon catalyst (7.5 g, 50% water content) were added to a solution of the product from part (d) (7.46 g, 9.57 mmol) in dioxane (125 ml) at room temperature under nitrogen. Hydrogen was bubbled through the solution for 30 minutes and then the reaction was stirred under hydrogen (1 atmosphere) for 11 hours. The reaction was filtered through a Celite® pad which was washed with water (about 100 ml) until no product could be detected in the eluent. The solution was frozen and lyophilized to give 4.5 g of a white solid. Purification by HPLC (reverse phase, methanol, water, trifluoroacetic acid), subsequent lyophilization, filtration through polyvinylpyridine with a water mobile phase, and final lyophilization proved 3.3 g of the desired product as a voluminous white solid. MS 426.2 $(M+H)^+$, 424.4 $(M-H)^-$; IR (KBr) 1777 cm^{-1}.

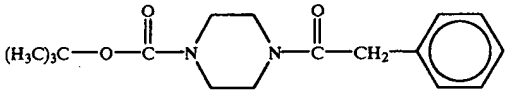
Anal. calc'd for $C_{18}H_{31}N_7O_5.1.56\ H_2O$: C, 47.66; H, 7.58; N, 21.62; O, 23.14. Found: C, 47.58, H, 7.37; N, 21.41.

EXAMPLE 33

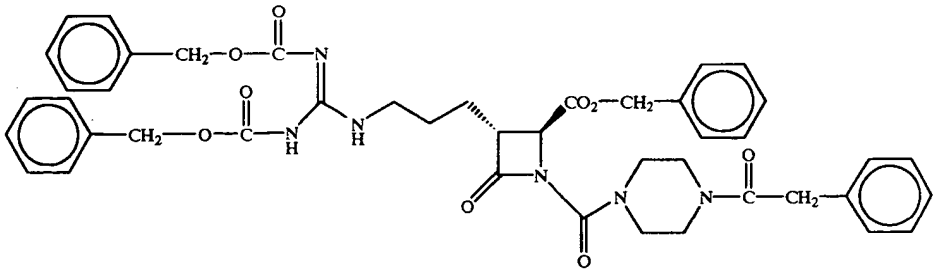


(homochiral)

a)



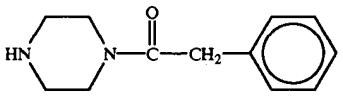
d)



Triethylamine (34 μl), 4-dimethylaminopyridine (30 mg) and a solution of the product from part (c) (74 mg, 0.28 mmol) in methylene chloride (2 ml) were added to a solution of the benzyl ester product from Example 1(c) (113 mg, 0.20 mmol) in methylene chloride (1 ml). The mixture was stirred

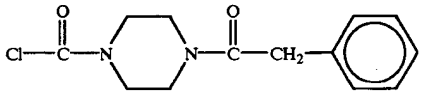
Following the procedure of Example 23(a) but substituting phenylacetyl chloride for the 3-phenylpropanoic acid chloride, the desired compound was obtained as a yellow solid.

b)



A mixture of the product from part (a) (2.68 mmol), trifluoroacetic acid (10 ml) and methylene chloride (10 ml) was stirred at room temperature for 90 minutes. TLC showed the completion of the reaction. The solvent was removed and 1N sodium hydroxide solution (10 ml) was added. The mixture was extracted with ethyl acetate (100 ml). The organic solution was washed with brine (20 ml), dried (magnesium sulfate), filtered and concentrated to give 536 mg of desired product as a colorless oil which was used without further purification. IR (film) 1630 cm^{-1}.

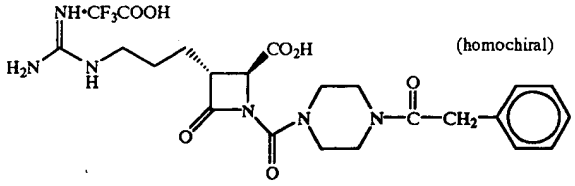
c)



A mixture of the product from part(b) (113 mg, 0.55 mmol) and triethylamine (92 μl) in methylene chloride (1 ml) was added to a solution of phosgene (351 μl, 20% in toluene) in methylene chloride (1 ml) at 0° C. The resultant mixture was stirred at 0° C. for 2 hours and worked-up according to the procedure of Example 23(c) to give 74 mg of the desired product as a yellow solid. IR(film) 1735 cm^{-1}, 1645 cm^{-1}.

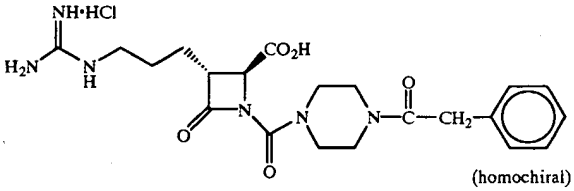
at room temperature for 2 hours. Analytical HPLC indicated that the reaction was complete. The reaction was quenched with the addition of 1N potassium sulfate (10 ml). The mixture was extracted with ethyl acetate (100 ml). The organic layer was washed with brine (15 ml), dried (magnesium sulfate) and concentrated to give the crude product as a colorless oil. Purification using flash chromatography (30–50% ethyl acetate/hexane) gave 73 mg of the desired product. MS $(M+H)^+$ 803.4, $(M-H)^-$ 801.5; IR (film) 1785 cm^{-1}, 1733 cm^{-1}, 1733 cm^{-1}, 1677 cm^{-1}, 1640 cm^{-1}.

e)

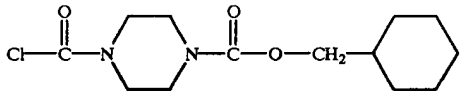


The product from part (d) (67 mg, 0.83 mmol) was deprotected and worked-up according to the procedure of Example 19(c) to give 5 mg of the desired product as a white solid. MS $(M+H)^+$+445.2, $(M-H)^-$ 443.4; IR (film) 1782 cm^{-1}, 1677 cm^{-1}.

EXAMPLE 34

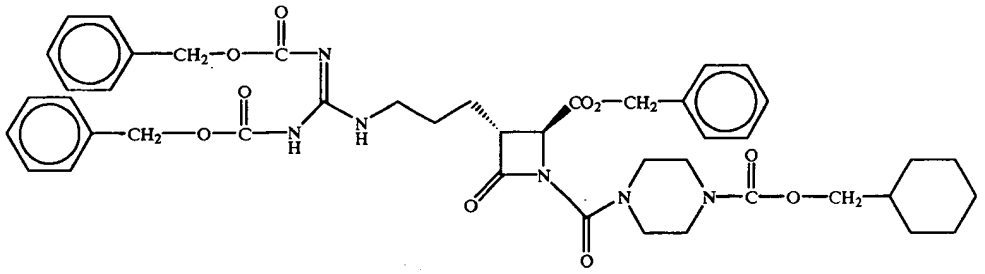


a)



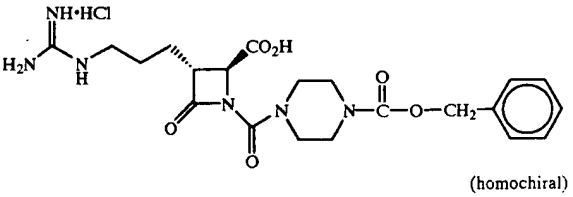
Following the procedure of Example 30 (a) through (d) but substituting cyclohexylmethanol for the cyclohexanol in step (a), the desired compound was obtained as a yellow oil. IR (film) 1743 cm^{-1}, 1702 cm^{-1}.

b)



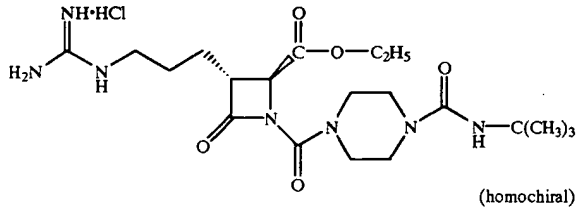
The product from part (a) is reacted with the benzyl ester product from Example 1(c) according to the procedure of Example 30 (e) to give the desired product as a colorless oil. IR (film) 1786 cm^{-1}, 1732 cm^{-1}, 1680 cm^{-1}, 1639 cm^{-1}.

c)



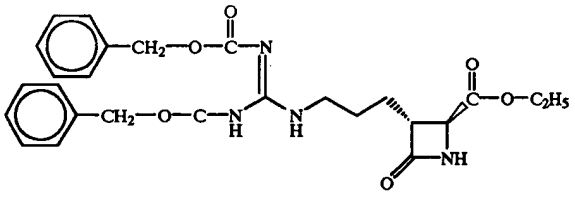
The product from part (b) was deprotected and worked-up as described in Example 21(d) to give the desired product as a white solid. MS $(M+H)^+$ 467.3, $(M-H)^-$ 465.5; IR (KBr) 1778 cm^{-1}, 1541 cm^{-1}.

EXAMPLE 35



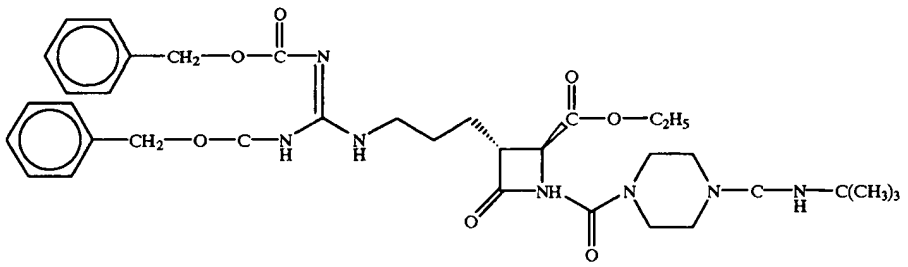
(homochiral)

a)

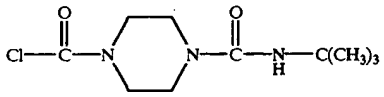


Cesium carbonate (14 mg, 0.042 mmol) was added to a stirred solution of the azetidinone product of Example 1(b) (40 mg, 0.083 mmol) and iodoethane (27 μl, 0.332 mmol) in dimethylformamide (200 μl) at room temperature. After 3 hours, the reaction mixture was partitioned between ethyl acetate and water containing a small amount of sodium thiosulfate. The organic phase was isolated, washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography to afford 33 mg of the desired product.

b)

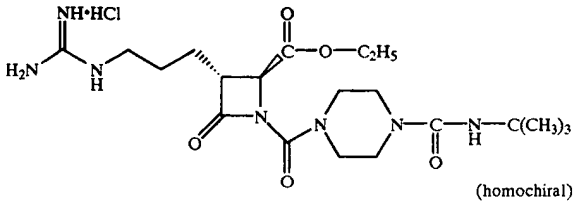


The product from part (a) (86 mg, 0.168 mmol) and the piperazinyl carbamoyl chloride of the formula:



(56 mg, 0.227 mmol) [prepared as described in Example 32(c)] were dissolved in methylene chloride (1.80 ml) and tetrahydrofuran (0.20 ml). Triethylamine (35 μl, 0.252 mmol) was added followed by 4-dimethylaminopyridine (4.0 mg, 0.034 mmol). After 48 hours the reaction was concentrated and the crude product was purified by silica gel chromatography to give 71 mg of the desired product.

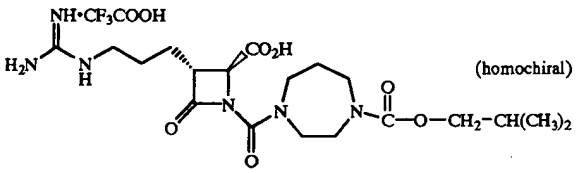
c)



(homochiral)

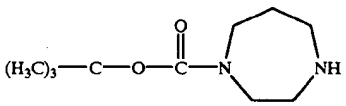
The product from part (b) was deprotected and worked-up according to the procedure described in Example 21(d) to give the desired product as a lyophilate. IR(KBr) 1788 cm^{-1}.

EXAMPLE 36



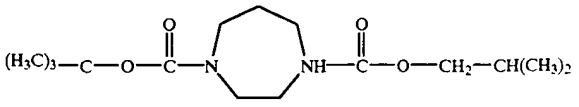
(homochiral)

a)



A solution of the di-tert-butyl dicarbonate (1.09, 4.99 mmol) and triethylamine (700 μl, 4.99 mmol) in tetrahydrofuran (15 ml) was added dropwise over 20 minutes to a solution of homopiperazine (400 mg, 4.99 mmol) in tetrahydrofuran (40 ml). The reaction mixture was stirred at room temperature for 2 hours. The mixture was then filtered and the filtrate was concentrated to give crude product as a colorless oil. Purification by flash chromatography (5% 2 N ammonia in methanol/methylene chloride) gave 410 mg of the desired product as a colorless oil. IR (film) 1691 cm^{-1}.

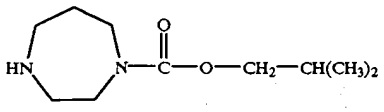
b)



Diisopropylethylamine (380 μl), 4-dimethylaminopyridine (35 mg) and a solution of isobutyl chloroformate (242 μl, 1.87 mmol) in methylene chloride (2 ml) were added to a solution of the product from part (a)

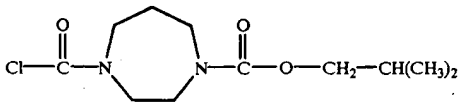
(374 mg, 1.87 mmol) in methylene chloride (2 ml). The mixture was stirred at 0° C. and warmed to room temperature overnight. The reaction was quenched with the addition of water (15 ml) and worked-up according to the procedure described in Example 28 (a) to give 508 mg of the desired product. IR (film) 1696 cm^{-1}.

c)



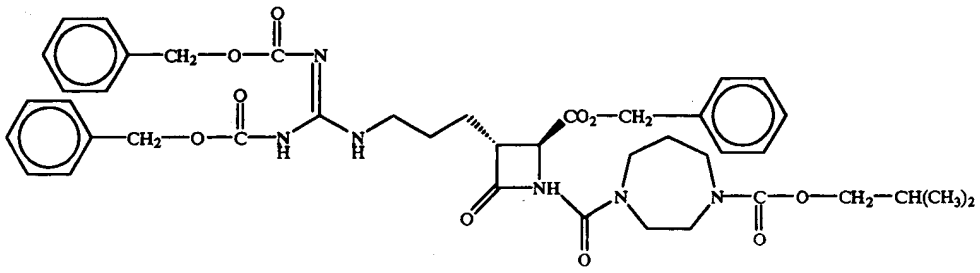
The product from part (b) (499 mg, 1.66 mmol) was treated with trifluoroacetic acid (2 ml) according to the procedure of Example 28(b) to give 324 mg of the desired product as a colorless oil which was used without further purification. IR(film) 1695 cm^{-1}.

d)



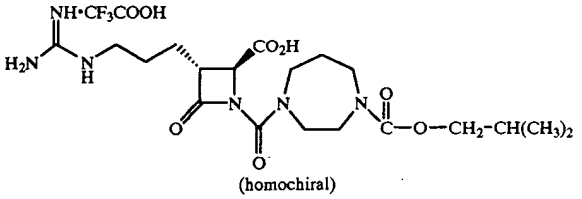
The product from part (c) (303 mg, 1.51 mmol) was reacted with phosgene in toluene (1.2 ml, 20%) at 0° C. according to the procedure of Example 28 (c) to give 298 mg of the desired product as a colorless oil. IR (film) 1737 cm^{-1}, 1697 cm^{-1}.

e)



Triethylamine (34 μl), 4-dimethylaminopyridine (10 mg) and a solution of the product from part (d) (62 mg, 0.24 mmol) in methylene chloride (2 ml) were added to a solution of the benzyl ester product from Example 1(c) (113 mg, 0.20 mmol) in methylene chloride (1 ml). The mixture was stirred at room temperature overnight. Analytical HPLC indicated that the reaction was complete. The reaction was quenched with the addition of 1N potassium bisulfate (15 ml) and worked-up according to the procedure of Example 28(d) to give, following purification, 92 mg of the desired product as a colorless oil. MS $(M+H)^+$ 799.4, $(M-H)^-$ 797.6.

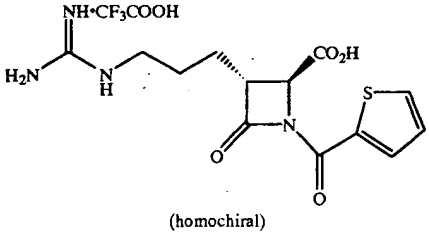
f)



(homochiral)

The product from part (e) (81 mg, 0.10 mmol) was deprotected and worked-up according to the procedure of Example 19(c) to give 5 mg of the desired product as a colorless glass. MS $(M+H)^+$ 441.3, $(M-H)^{-1}$ 439.4; IR film) 1784 cm^{-1}, 1665 cm^{-}.

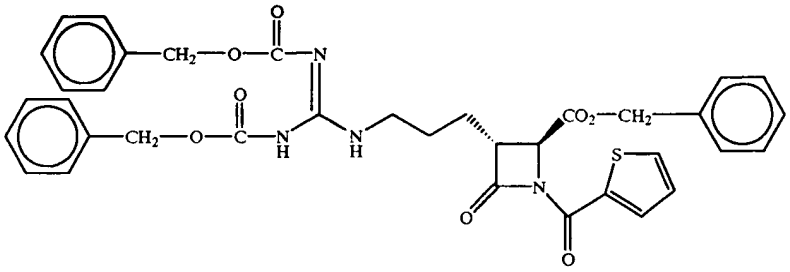
EXAMPLE 37



(homochiral)

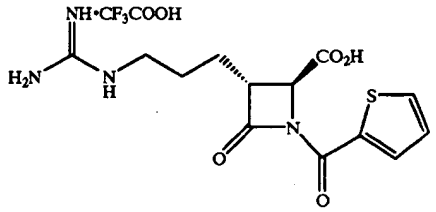
-continued

a)



Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 154 μl, 0.15 mmol) was added dropwise to a −78° C. solution of the benzyl ester product from Example 1(c) (80 mg, 0.14 mmol) in tetrahydrofuran (2 ml). The mixture was stirred at −78° C. for 40 minutes. A solution of 2-thiophenecarbonyl chloride (34 μl, 0.30 mmol) in tetrahydrofuran was added. The reaction was stirred at −78° C. for an additional 6 hours and was stored in a freezer (−50° C.) overnight. Analytical HPLC indicated the reaction was complete. The reaction mixture was quenched by the addition of saturated ammonium chloride solution (5 ml). The mixture was extracted with ethyl acetate (3×15 ml). The organic layers were combined and washed with brine (2×10 ml), dried (magnesium sulfate), filtered and concentrated to give the crude product which was purified by flash chromatography (silica, 20–30% ethyl acetate/hexane) to give 57 mg of the desired product as a white solid. MS $(M+H)^+$ 683.7, $(M-H)^-$ 681.6; IR (KBr) 1796 cm^{-1}, 1734 cm^{-1}, 1640 cm^-.

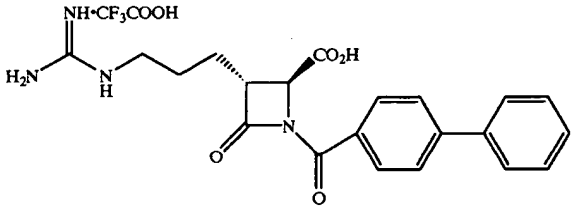
b)



(homochiral)

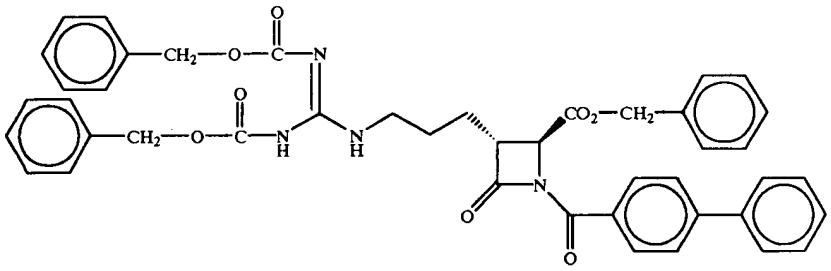
The product from part (a) (53 mg, 0.078 mmol) was deprotected and worked-up according to the procedure of Example 19(c) to give 11 mg of the desired product as a white powder. MS $(M+H)^+$ 324.9, $(M-H)^-$ 323.1.

EXAMPLE 38



(homochiral)

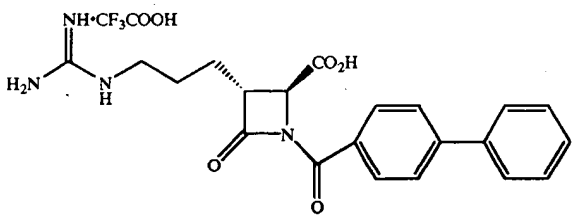
a)



Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 131 μl, 0.131 mmol) was added to a solution of the benzyl ester product from Example 1(c) (73.1 mg, 0.127 mmol) in dry tetrahydrofuran (2 ml) under nitrogen at −78° C. The reaction mixture was stirred at −78° C. for 30 minutes then 4-biphenylcarbonyl chloride (29 mg, 0.134 mmol) was added in a single portion. The reaction mixture was stirred at −78° C. for 5 minutes and then at −15° C. for

15 minutes. 1N HCl (1 ml) was added followed immediately by ethyl acetate (3 ml). The resulting biphasic solution was stirred vigorously while warming to room temperature. The organic phase was separated, dried over magnesium sulfate, filtered and concentrated to leave a light yellow residue. Purification by flash chromatography (silica gel, 0–30% ethyl acetate in hexane) gave 28 mg of the desired product. IR (film) 1797 cm^{-1}; MS 753.1 $(M+H)^+$.

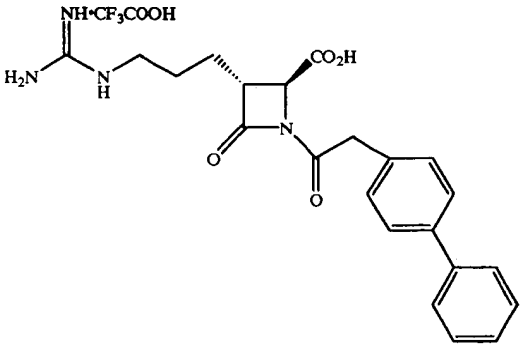
b)



(homochiral)

The product from part (a) (28 mg, 0.037 mmol) was deprotected and worked-up according to the procedure of Example 19(c) to give 11.8 mg of the desired product as a lyophilate. IR (film) 1788 cm^{-1}; MS 395.1 $(M+H)^+$, 393.3 $(M-H)^-$.

EXAMPLE 39

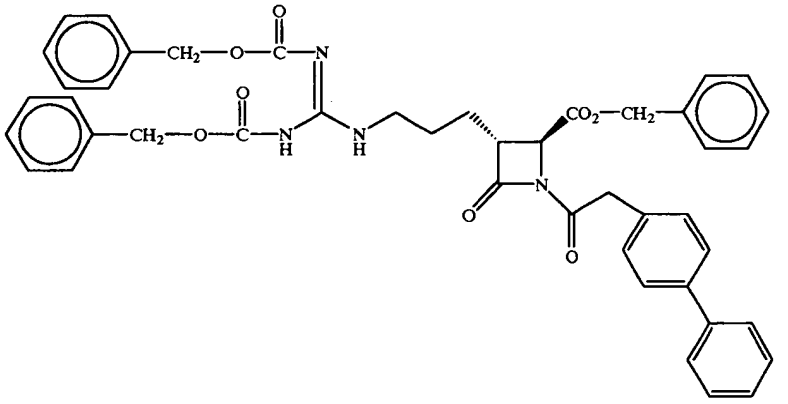


(homochiral)

a) Biphenylacetyl chloride

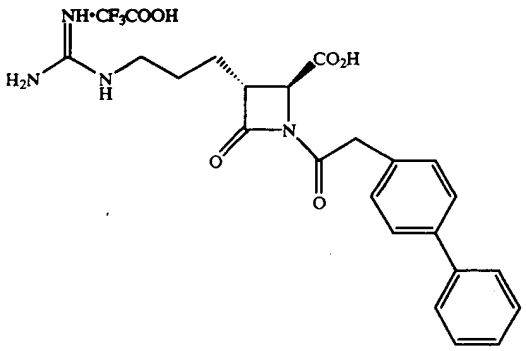
Oxalyl chloride (246 μl, 2.86 mmol) and one drop of dimethylformamide were added dropwise to a suspension of biphenylacetic acid (30 mg, 1.41 mmol) in methylene chloride (10 ml). The mixture was stirred at room temperature for 20 minutes. The solvent was evaporated and the residue was coevaporated with toluene twice to give 310 mg of the title product as a yellow solid.

b)



Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 315 μl, 0.31 mmol) was added dropwise to a −78° C. solution of the benzyl ester product of Example 1(c) (120 mg, 0.21 mmol) in tetrahydrofuran (3 ml). The mixture was stirred at −78° C. for 2.5 hours. A solution of biphenylacetyl chloride (58 mg, 0.25 mmol) in tetrahydrofuran (1 ml) was added. The reaction mixture was stirred at −78° C. for an additional 2.5 hours and was stored in afreezer (−50° C.) overnight. The reaction mixture was partitioned between ethyl acetate (50 ml) and water (15 ml). The organic layer was separated and washed with brine (20 ml), dried (magnesium sulfate), filtered and concentrated to give the crude product which was purified by flash chromatography (silica, 20–30% ethyl acetate/hexane) to give 22 mg of the desired product as a yellow solid. MS $(M+H)^+$ 767.1; IR (KBr) 1796 cm^{-1}, 1730 cm^{-1}, 1640 cm^{-1}.

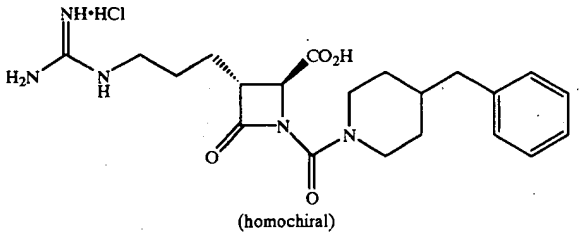
c)



(homochiral)

The product from part (b) (40 mg, 0.052 mmol) was deprotected and worked-up according to the procedure of Example 19(c) to give 11 mg of the desired product as a white fluffy powder; MS:$(M+H)^+$ 409.2,$(M-H)^-$ 407.2; IR (KBr) 1782 cm^{-1}, 1684 cm^{-1}, 1645 cm^{-1}.

EXAMPLE 40

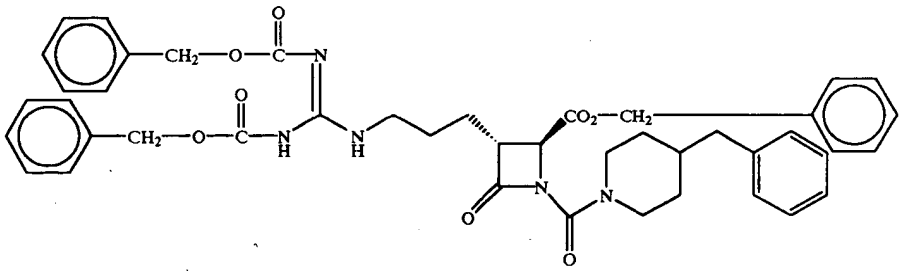


(homochiral)

a) 4-Benzylpiperidinylcarbonyl chloride

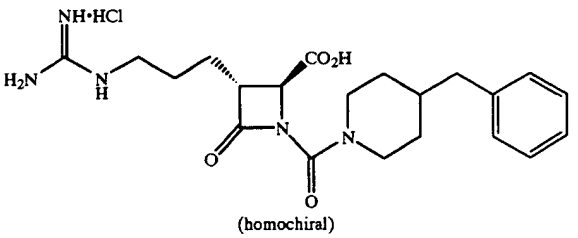
4-Benzylpiperidine (0.5 g, 2.86 mmol) was added to a mixture of phosgene (3.8 ml of 20% phosgene in toluene solution, 7.13 mmol). The mixture was stirred at 0° C. for 1 hour. The reaction mixture was partitioned between water (25 ml) and ethyl acetate (2×25 ml). The organic phase was washed with 1N HCl (1×40 ml), saturated sodium chloride (1×50 ml), dried over sodium sulfate and concentrated to give a yellow oil. Purification by flash column chromatography (silica gel, 0–10% ethyl acetate/hexane) gave 0.47 g of title product. IR (film) 1733.1 cm^{-1}.

b)



Triethylamine (17 mg, 0.165 mmol) and 4-dimethylaminopyridine (6–8 crystals) were added to a cooled solution of the benzyl ester product from Example 1(c) (63 mg, 0.11 mmol) in methylene chloride (2 ml) at 0° C. 4-Benzylpiperidinylcarbonyl chloride (39 mg, 0.165 mmol) was added and the mixture was stirred at 0° C. for 45 minutes followed by stirring at room temperature for 2.5 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash chromatography (silica gel, 0–30% ethyl acetate/hexane) gave 68 mg of the desired product as a colorless oil. MS 774.2 $(M+H)^+$, 772.4 $(M-H)^-$; IR (film) 1785.1, 1732.0, 1674.2, 1639.3 cm^{-1}.

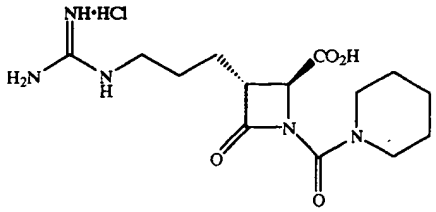
c)



(homochiral)

The product from part (b) (65 mg, 0.084 mmol) was deprotected and worked-up as described in Example 21(d) to give 39 mg of the desired product as a white lyophilate. MS 416.2 $(M+H)^+$, 414 $(M-H)^-$; IR(KBr) 1784, 1657 cm^{-1}.

EXAMPLE 41

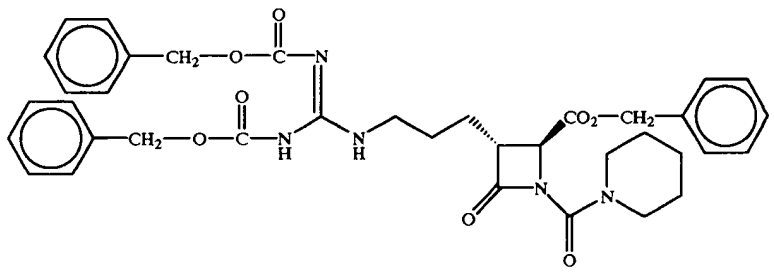


(homochiral)

a) N-Piperidinylcarbonyl chloride

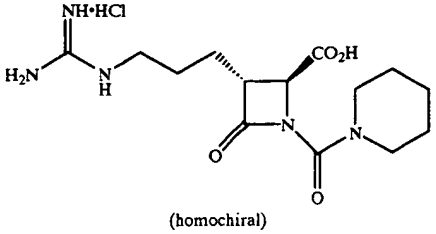
Piperidine (0.3 g, 3.52 mmol) was added to a mixture of phosgene (4.7 ml of 20% phosgene in toluene solution, 8.81 mmol) in methylene chloride (5 ml) at 0° C. The mixture was stirred at 0° C. for 1 hour. The reaction mixture was evaporated in vacuo. The residue was suspended in ether, filtered and the eluents were condensed to obtain a yellow oil. Purification by flash column chromatography (silica gel, 0–20% ethyl acetate/hexane) gave 0.162 g of the title product. IR (film) 1738.9 cm^{-1}.

b)



Triethylamine (32 mg, 0.314 mmol) and 4-dimethylaminopyridine (10–15 crystals) were added to a cooled solution of the benzyl ester product from Example 1(c) (120 mg, 0.21 mmol) in methylene chloride (1 ml) at 0° C. N-Piperidinylcarbonyl chloride (46 mg 0.314 mmol) was added and the mixture was stirred at room temperature for 16 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash column chromatography (silica gel, 0–20% ethyl acetate/hexane) gave 95 mg of the desired product as a colorless gum. MS 684.3 $(M+H)^+$, 682.5 $(M-H)^-$; IR (film) 1783.9, 1731.0 cm^{-1}.

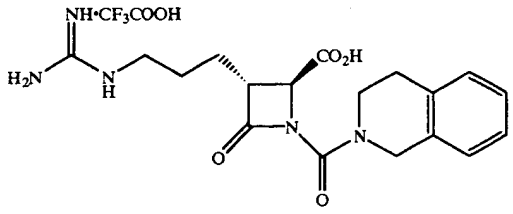
c)



(homochiral)

The product from part (b) (65 mg, 0.089 mmol) was deprotected and worked-up as described in Example 21(d) to give 11 mg of the desired product as a colorless glass. MS 326.3 (M+H+), 324.3, $(M-H)^-$.

EXAMPLE 42

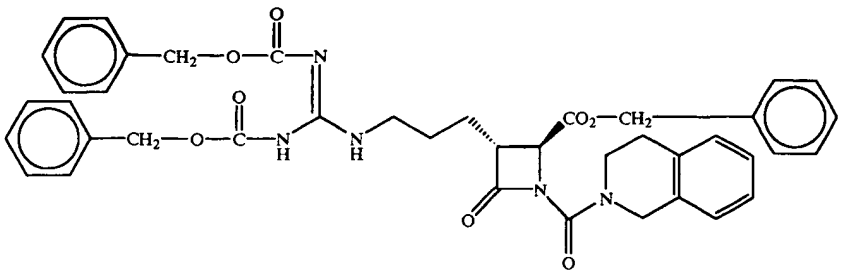


(homochiral)

a) 1,2,3,4-Tetrahydroisoquinolinylcarbonyl chloride

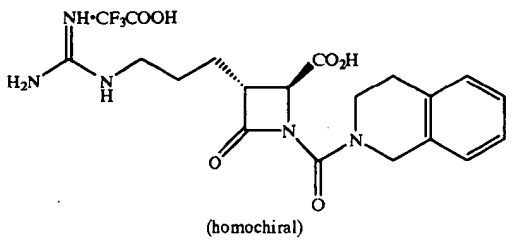
1,2,3,4-Tetrahydroisoquinoline (0.5 g, 3.76 mmol) was added to a cooled mixture of phosgene (5 ml of 20% phosgene in toluene solution, 9.4 mmol) in methylene chloride (5 ml) at 0° C. The mixture was stirred at 0° C. for 1 hour. The reaction mixture was evaporated in vacuo. The residue was suspended in ethyl ether, filtered and the eluents were condensed to give a pale pink oil. Purification by flash column chromatography (silica gel, 0–10% ethyl acetate/hexane) gave 0.586 g of the desired product. IR (film) 1735.3 cm^{-1}.

b)



Triethylamine (20 mg, 0.2 mmol) and 4-dimethylaminopyridine (8–10 crystals) were added to a cooled solution of the benzyl ester product from Example 1(c) (77 mg, 0.135 mmol) in methylene chloride (2 ml) at 0° C. 1,2,3,4-Tetrahydroisoquinolinylcarbonyl chloride (39 mg, 0.2 mmol) was added and the mixture was stirred at room temperature for 2.5 hours. The mixture was then evaporated in vacuo. Purification of the residue by flash column chromatography (silica gel, 0–30% ethyl acetate/hexane) have 66 mg of the desired product as a colorless oil. MS 732.3 (M+H+), 730.7, $(M-H)^-$; IR (film) 1790.2, 1732.0, 1673.8, 1638.9 cm^{-1}.

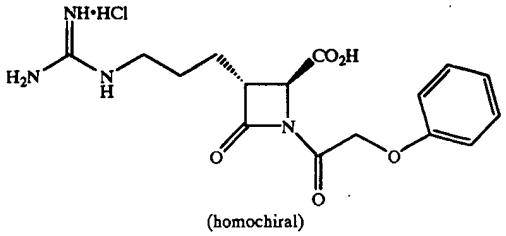
c)



(homochiral)

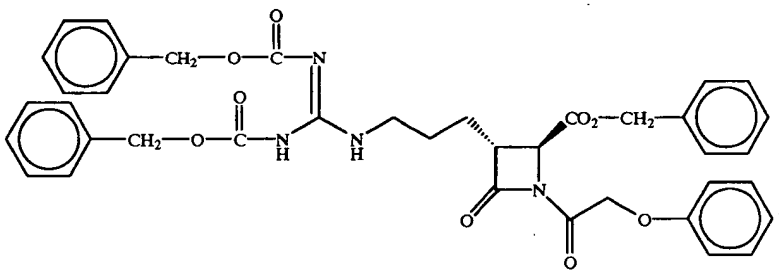
The product from part(b) (65 mg, 0.089 mmol) was deprotected and worked-up as described in Example 19(c) to give 33 mg of the desired product as a white foam. MS 374.2 $(M+H)^+$, 372.4 $(M-H)^-$; (film) 1788.0, 1668.0 cm^{-1}.

EXAMPLE 43



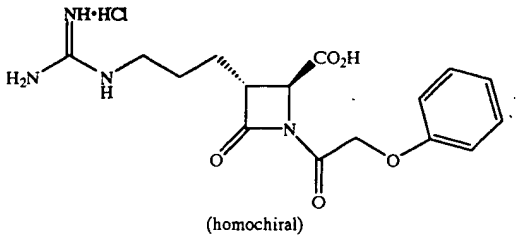
(homochiral)

a)



Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 180 μl, 0.18 mmol) was added dropwise to a −78° C. solution of the benzyl ester product from Example 1(c) (92 mg, 0.16 mmol) in tetrahydrofuran (2 ml). The mixture was stirred at −78° C. for 1 hour. Phenoxyacetyl chloride (24 μl) was added. The reaction mixture was stirred at −78° C. for an additional 20 minutes and was stored in a freezer (−50° C.) overnight. Analytical HPLC indicated that the reaction was not completed. Another 24 μl of phenoxyacetyl chloride was added and the mixture was stirred at −78° C. for an additional 3.5 hours. The reaction mixture was quenched by the addition of water (10 ml). This was extracted with ethyl aceate (3×20 ml). The organic layers were combined and washed with brine (2×10 ml), dried (magnesium sulfate), filtered and concentrated to give the crude product which was purified by flash chromatography (silica, 30% ethyl acetate/hexane) to give 65 mg of the desired product as a colorless oil. MS $(M+H)^+$ 707.1, $(M-H)^-$ 705.4; IR (KBr) 1798 cm^{-1}, 1640 cm^{-1}.

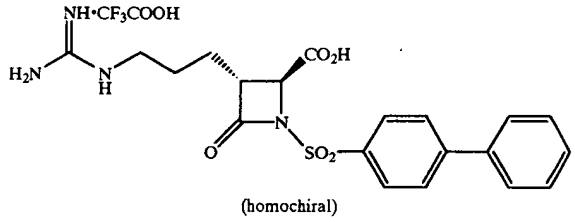
b)



(homochiral)

The product from part (a) (63 mg, 0.089 mmol) was deprotected and worked-up as described in Example 21(d) to give 31 mg of the desired product as a white powder: MS: $(M+H)^+$ 349.0, $(M-H)^-$ 347.2; IR (KBr) 1800 cm^{-1}, 1723 cm^{-1}, 1649 cm^{-1}.

EXAMPLE 44



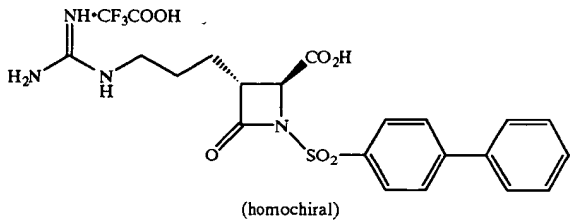
(homochiral)

a) 4-Biphenylsulfonyl chloride

Sulfuryl chloride (392 μl, 4.74 mmol) was added dropwise to a 0° C. suspension of triphenylphosphine-resin (1.58 g) in methylene chloride (10 ml). A solution of 4-biphenylsulfonic acid (400 mg, 1.58 mmol) and triethylamine (220 μl) in methylene chloride (5 ml) was added. The mixture was stirred at room temperature for 6 hours and stored at 5° C. for 3 days. This was filtered and the filtrate was evaporated. The residue was coevaporated with toluene twice to give the crude product as a white solid. Purification of the crude product by chromatography (silica, methylene chloride) gave 180 mg of the title product as a white solid.

Sodium bis(trimethylsilyl)amide (1.0 M in tetrahydrofuran, 255 μl, 0.26 mmol) was added dropwise to a −78° C. solution of the benzyl ester product from Example 1(c) (95 mg, 0.17 mmol) in tetrahydrofuran (3 ml). The mixture was stirred at −78° C. for 40 minutes. A solution of 4-biphenylsulfonyl chloride (64 mg, 0.26 mmol) in tetrahydrofuran (1 ml) was added. The reaction was stirred at −78° C. for an additional 20 minutes and was stored in a freezer (−50° C.) overnight. Analytical HPLC indicated the reaction was completed. The reaction was quenched with the addition of 1N potassium bisulfate (20 ml). The mixture was extracted with ethyl acetate (2×50 ml). The organic layers were combined and washed with brine (20 ml), dried (magnesium sulfate), filtered and concentrated to give the crude product (158 mg) as a yellow oil. Purification of the crude product by flash chromatography (silica, 30% ethyl acetate/hexane) gave 76 mg of the desired product as a colorless oil. MS $(M+H)^+$ 789.0.

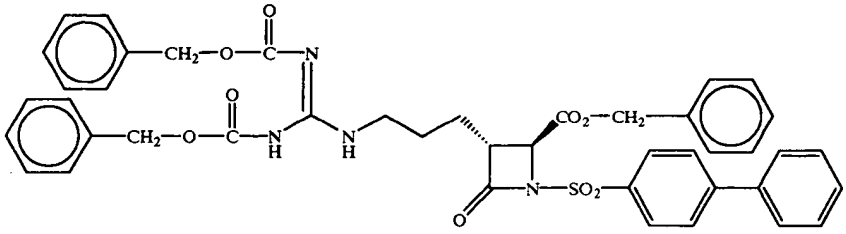
c)



(homochiral)

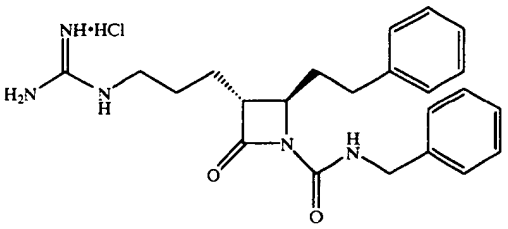
The product from part (b) (70 mg, 0.088 mmol) was deprotected and worked-up according to the procedure of Example 19(c) to give 17 mg of the desired product as a white fluffy powder. MS $(M+MeOH+H)^+$ 463.2, $M+MeOH-H)^-$ 461.5; IR (KBr) 1773 cm^{-1}, 1665 cm^{-1}, 1595 cm^{-1}.

b)



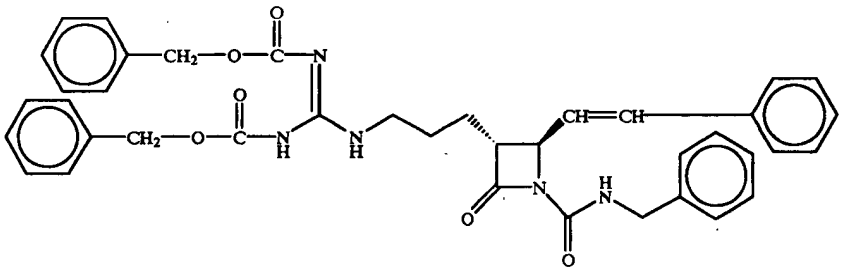
EXAMPLE 45

trans-3-[3-[(Aminoiminomethyl)amino]propyl]-2-oxo-4-(2-phenylethyl)-N-(phenylmethyl)-1-azetinecarboxamide, monohydrochloride



(racemate)

a)



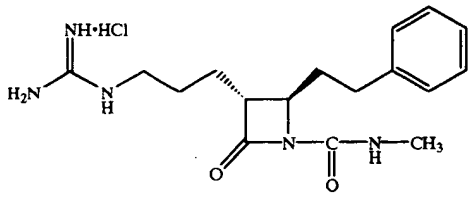
A solution of trans-4-(2-phenylethenyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (130 mg, 0.2 mmol, prepared as described in Example 3 of Han U.S. Pat. No. 5,037,819) in tetrahydrofuran (1.5) was cooled to −78° C. under an argon atmosphere. A 1 M solution of sodium bis(trimethylsilyl)amide (0.21 ml) in tetrahydrofuran was added and the mixture stirred for 15 minutes. Benzylisocyanate (40 mg, 0.3 mmol) was added dropwise and the mixture was allowed to warm to room temperature and stirred for 1 hour. The reaction was diluted with aqueous 10% potassium hydrogen sulfate solution (10 ml) and extracted with ethyl acetate (3×10 ml); the combined organic phase was washed with water (25 ml), brine (25 ml) and dried over sodium sulfate. The solution was filtered and the solvent evaporated to give an oil. The residue was purified by flash column chromatography (silica, ethyl acetate:hexane, 2:3) yielding 69 mg of the desired product as a colorless oil. MS $(M+H)^+$ 674.

b) trans-3-[3-(Aminoiminomethyl)amino]propyl]-2-oxo-4-(2-phenylethyl)-N-(phenylmethyl)-1-azetinecarboxamide, monohydrochloride

A solution of the product from part (a) (67 mg, 0.1 mmol) in dioxane (1.5 ml) containing aqueous 1N HCl (0.15 ml) and 10% palladium on carbon catalyst was stirred under a hydrogen atmosphere for 2 hours. The reaction was filtered and lyophilized to give 66 mg of the titled product as a colorless solid; m.p. 145–154° C.(dec). MS $(M+H)^+$ 408; IR (KBr) 1761 cm^{-1}.

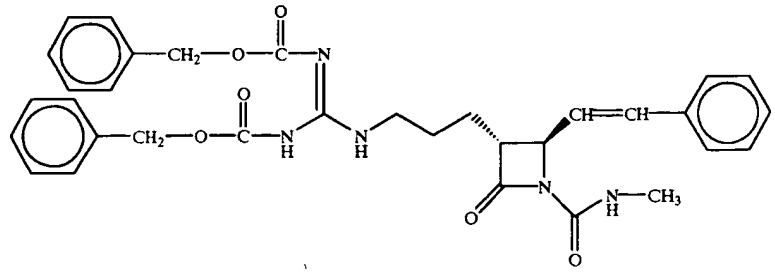
EXAMPLE 46

trans-3-[3-[(Aminoiminomethyl)amino]propyl]-N-methyl-2-oxo-4-(2-phenylethyl)-1-azetinecarboxamide, monohydrochloride



(racemate)

a)

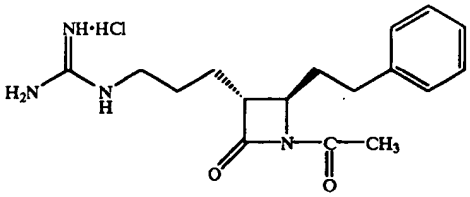


Following the procedure of Example 45(a) but substituting methylisocyanate (23 mg, 0.5 mmol) for the benzylisocyanate, the desired product (80 mg) was obtained as a colorless oil. MS $(M+H)^+$ 598.

b) trans-3-[3-[(Aminoiminomethyl)amino]propyl]-N-methyl-2-oxo-4-(2-phenylethyl)-1-azetinecarboxamide, monohydrochloride

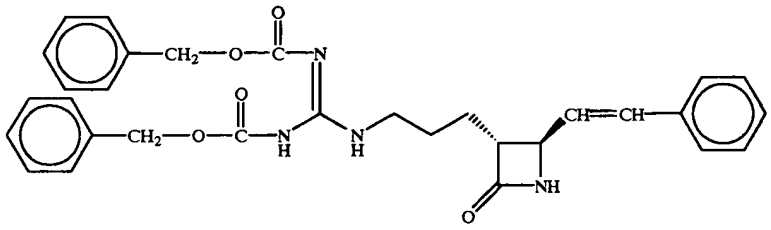
b) The product from part (a) (77 mg, 0.13 mmol) was deprotected and worked-up as described in Example 45 (b) to give 42 mg of the titled 10 product as a colorless solid; m.p. 138–146° (dec). MS $(M+H)^+$ 332; IR(KBr) 1761 cm^{-1}.

EXAMPLE 47



[Chiral(+)]

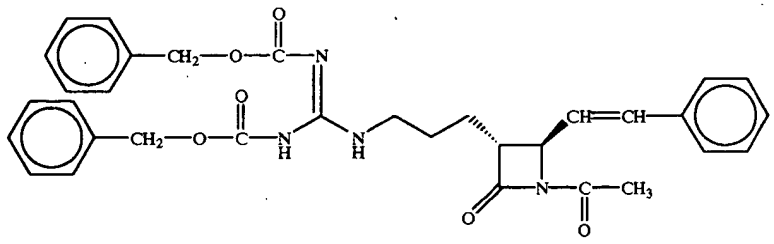
a)



[Chiral(-)]

b) trans-4-(2-Phenylethenyl)-3-[3-[N,N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone was separated into enantiomerically pure (–) isomer and (+) isomer on a Chiralpak-AD® prep-column eluting with 30% 2-propanol/hexane.

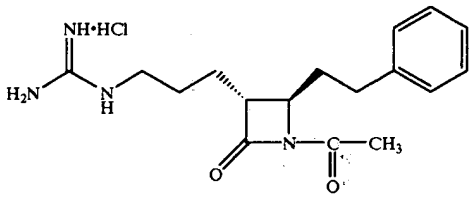
b)



A solution of chiral(–)-trans-4-(2-phenylethenyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (216 mg, 0.40 mmol) in tetrahydrofuran (2.0 ml) was cooled to –78° C. under an argon atmosphere. A 1M solution of sodium bis(trimethylsilyl)amide (0.44 ml) in tetrahydrofuran was added and the mixture was stirred for 15 minutes. Acetyl chloride (32.2 mg, 0.41 mmol) was added dropwise and the mixture was warmed to room temperature and stirred for 1 hour. The reaction was diluted with aqueous 10% potassium hydrogen sulfate solution (10 ml) and extracted with ethyl acetate (3x10 ml); the combined organic phase was washed with water (25 ml), brine (25 ml) and dried over sodium sulfate. The solution was filtered and the solvent evaporated to give an oil. The residue

was purified by flash chromatography (silica, ethyl acetate:hexane, 1:3) to give 150 mg of the desired product as a colorless oil. MS $(M+H)^+$ 583.

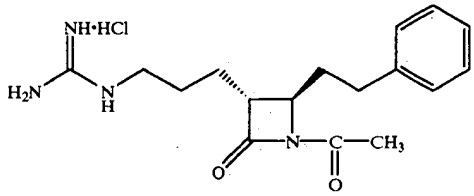
c)



[Chiral(+)]

A solution of the product from part (b) (140 mg, 0.258 mmol) in dioxane (3.5 ml) containing 1N HCl (0.3 ml) and 10% palladium on carbon catalyst (60 mg) was stirred under a hydrogen atmosphere for 1 hour. The reaction was filtered and lyophilized to give 64 mg of the desired product as a colorless solid. MS $(M+H)^+$ 317; IR(KBr) 1782 cm^{-1}; $[\alpha]_D$=+18° (c=1, methanol).

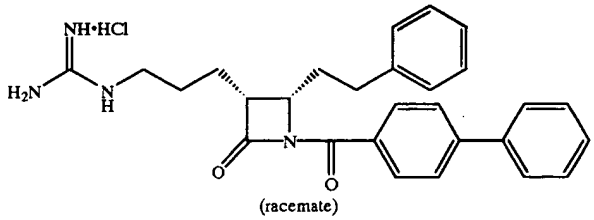
EXAMPLE 48



[Chiral(-)]

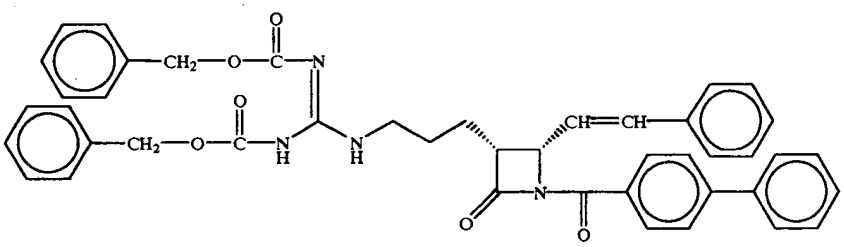
Following the procedure of Example 47(b) and (c) but employing chiral(+)-trans-4-(2-phenylethenyl)-3-[3-[N',N"-bis(carbobenzyloxy)-guanidino]propyl]-2-azetidinone, the desired product was obtained.

EXAMPLE 49



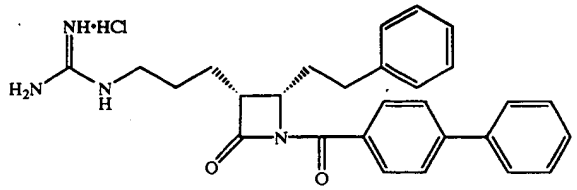
(racemate)

a)



A solution of cis-4-(2-phenylethenyl)-3-[3-[N',N"-bis (carbobenzyloxy)guanidino]propyl]-2-azetidinone (300 mg, 0.555 mmol, prepared as described in Example 3 of Han U.S. Pat. No. 5,037,819) in tetrahydrofuran (2.5 ml) was cooled to −78° C. under an argon atmosphere. A 1 Msolution of sodium bis(trimethylsilyl)amide in tetrahydrofuran (0.8 ml) was added and the mixture stirred for 15 minutes. 4-Biphenylcarbonyl chloride (180 mg, 0.832 mmol) was added dropwise and the mixture was allowed to warm to room temperature and stirred for 4 hours. The reaction was diluted with aqueous 10% potassium hydrogen sulfate solution (15 ml) and extracted with ethyl acetate (3×15 ml); the combined organic phase was washed with water (25 ml), brine (25 ml) and dried over sodium sulfate. The solution was filtered and the solvent evaporated to give an oil. The residue was purified by flash column chromatography (silica, ethyl acetate:hexane, 1:4) yielding 320 mg of the desired product as a colorless solid. MS (M+H)+=721.

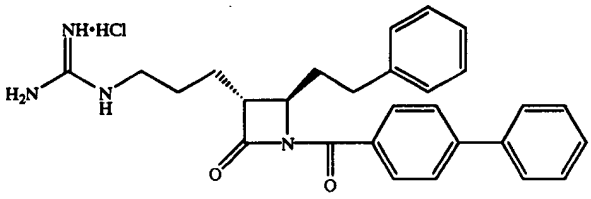
b)



(racemate)

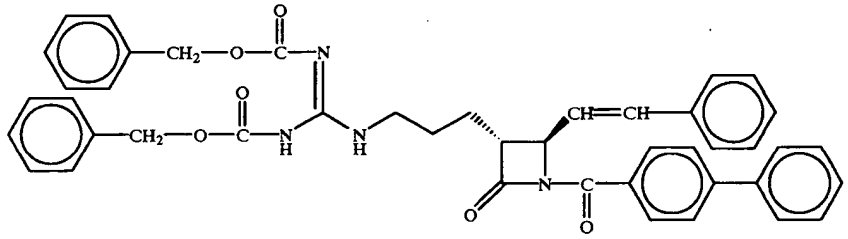
A solution of the product from part (a) (300 mg, 0.48 mmol) in dioxane (3 ml) containing aqueous 1N HCl (0.65 ml) and 10% palladium on carbon catalyst (150 mg) was stirred under a hydrogen atmosphere for 2 hours. The reaction was filtered and lyophilized to give 178 mg of the desired product as a colorless solid. MS $(M+H)^+$ 455; IR (KBr) 1782 cm^{-1}.

EXAMPLE 50



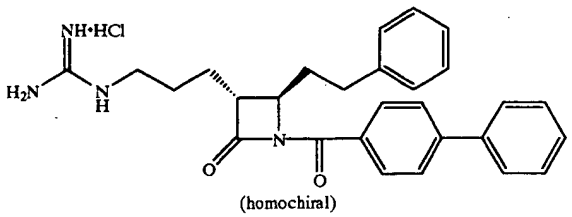
(homochiral)

a)



A solution of chiral-(+)-trans-4-(2-phenylethenyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (264 mg, 0.49 mmol) in tetrahydrofuran (2.5 ml) was cooled to −78° C. under an argon atmosphere. A 1M solution of sodium bis(trimethylsilyl)amide (0.74 ml) in tetrahydrofuran was added and the mixture stirred for 15 minutes. 4-Biphenylcarbonyl chloride (163 mg, 0.75 mmol) was added dropwise and the mixture was allowed to warm to room temperature and stirred for 4 hours. The reaction was diluted with aqueous 10% potassium hydrogen sulfate solution (15 ml) and extracted with ethyl acetate (3×15 ml); the combined organic phase was washed with water (25 ml), brine (25 ml) and dried over sodium sulfate. The solution was filtered and the solvent evaporated to give an oil. The residue was purified by flash column chromatography yielding 290 mg of the desired product as a colorless solid. MS $(M+H)^+$ 721.

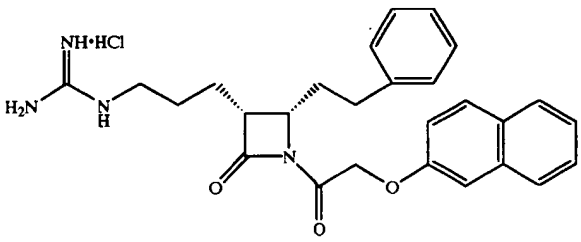
b)



(homochiral)

The product from part (a) (280 mg, 0.4 mmol) was deprotected and worked up as described in Example 49(b) to give 172 mg of the desired product as a colorless solid. MS $(M+H)^+$ 455; IR(KBr) 1782 cm^{-1}; $[\alpha]_{22}$=+12° (c=1, methanol).

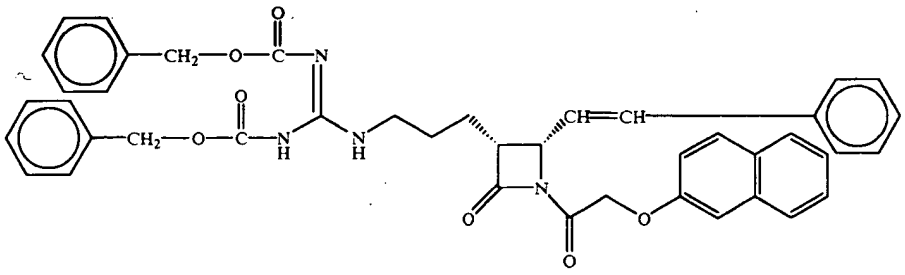
EXAMPLE 51



(racemate)

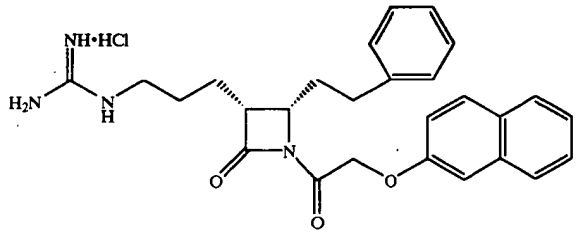
-continued

a)



Following the procedure of Example 49(a) but substituting (2-naphthyloxy)acetyl chloride for the 4-biphenylcarbonyl chloride, the desired product (134 mg) was obtained as a colorless solid. MS $(M+H)^+$ 725.

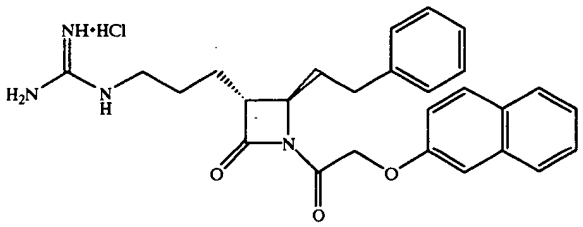
b)



(racemate)

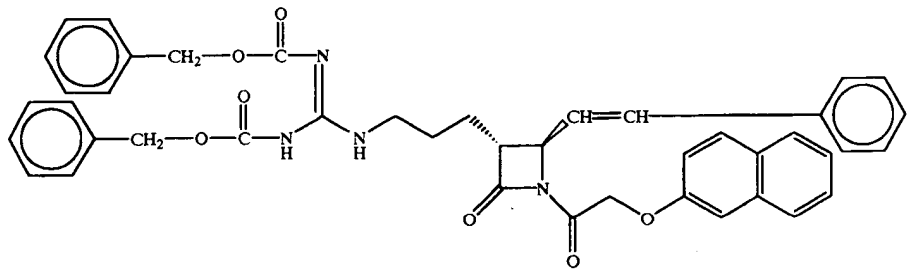
The product from part (a) (125 mg, 0.17 mmol) was deprotected and worked-up as described in Example 49(b) to give 76 mg of the desired product as a colorless solid. MS (M+H)+=459; IR(KBr) 1780 cm^{-1}.

EXAMPLE 52



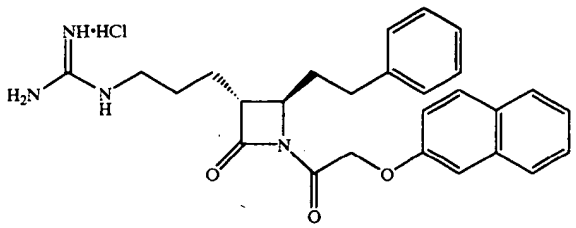
(homochiral)

a)



Following the procedure of Example 50(a) but substituting (2-naphthyloxy)acetyl chloride for the 4-biphenylcarbonyl chloride, the desired product (216 mg) was obtained as a colorless solid. MS $(M+H)^+$ 725.

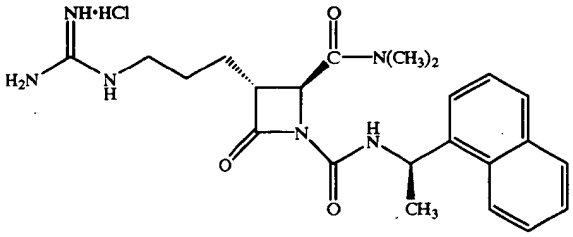
b)



(homochiral)

The product from part (a) (200 mg, 0.276 mmol) was deprotected and worked-up as described in Example 49(b) to give 108 mg of the desired product as a colorless solid. MS $(M+H)^+$ 459; IR (KBr) 1780 cm^{-1}; $[\alpha]_{22-+}18°$ (c=1, methanol).

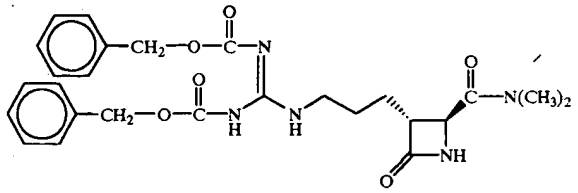
EXAMPLE 53



(homochiral)

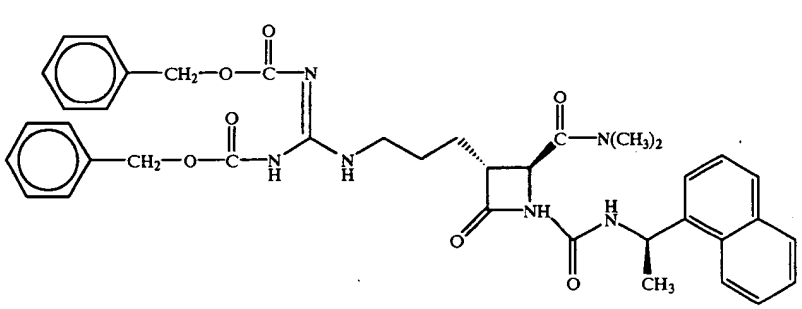
-continued

a)



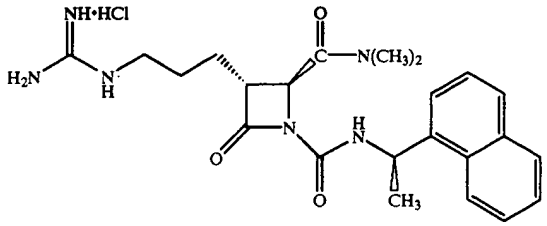
A solution of the carboxylic acid product from Example 1(b) (140 mg, 0.288 mmol) in tetrahydrofuran (2.5 ml) was cooled to −20° C. under an argon atmosphere and N-methylmorpholine (32.1 mg, 0.317 mmol) was added. A 2 M solution of dimethylamine (1.1 eq) in tetrahydrofuran was added followed by the addition of benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (140 mg, 0.317 mmol). The reaction was stirred at −20° C. for 24 hours, poured into 5% potassium hydrogen sulfate solution and extracted with ethyl acetate. The ethyl acetate extract was washed with water, brine and dried over sodium sulfate. The solvents were evaporated and the crude residue was purified by silica chromatography eluting with ethyl acetate yielding 56 mg of the desired product as a colorless solid. MS $(M+H)^+$ 510.

b)



A solution of the product from part (a) (48 mg, 0.094 mmol) in tetrahydrofuran (0.4 ml) was cooled to −78° C. under an argon atmosphere. Sodium bis(trimethylsilyl) amide (1 M, 1.5 eq.) was added and the mixture was stirred for 30 minutes. (R)-(−)-1-(1-Naphthenyl)-ethyl isocyanate (27.2 mg, 0.141 mmol) was added. The mixture was stirred at −78° C. for 30 minutes and then allowed to warm to room temperature and stir for 4 hours. The reaction was poured into 5% potassium hydrogen sulfate solution and extracted with ethyl acetate. The ethyl acetate extract was washed with water, brine and dried over sodium sulfate. The solvents were evaporated and the crude residue purified on silica by chromatography eluting with ethyl acetate:hexane (3:2) yielding 25 mg of the desired product as a colorless glass-like residue. MS $(M+H)^+$ 707.

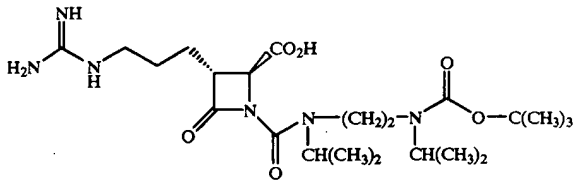
c)



(homochiral)

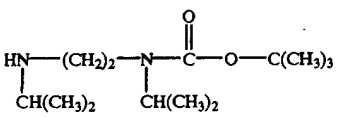
A solution of the product from part (b) (24 mg, 0.03 mmol) in dioxane (1 ml) containing HCl (1.5 eq.) was stirred under a hydrogen atmosphere with 10% palladium on carbon catalyst (12 mg) for 2 hours. The reaction was filtered and the solvents lyophilized to yield 14 mg of the desired product as a colorless solid; MS $(M+H)^+$ 439; $[\alpha]_D$=+12° (c=1, methanol).

EXAMPLE 54



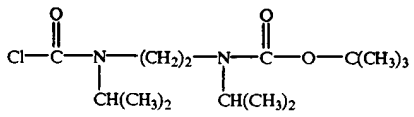
(homochiral)

a)



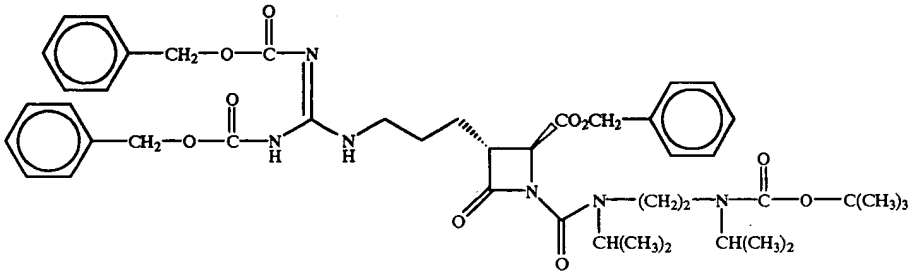
A mixture of di-tert-butyl dicarbonate (1.51 g, 6.9 mmol) and triethylamine (0.7 g, 6.9 mmol) in anhydrous tetrahydrofuran (3 ml) was added over 20 minutes to a solution of N,N'-diisopropylethylenediamine (1.0 g, 6.9 mmol) in anhydrous tetrahydrofuran (2 ml). The mixture was stirred at room temperature for 2 hours. The mixture was then filtered and washed with methylene chloride. The filtrate and washings were condensed to obtain a colorless oil. Purification by flash column chromatography (silica gel, 1–5% 2M ammonia in methanol/methylene chloride) gave 50 mg of the desired product as a colorless oil. MS 245.2 $(M+H)^+$.

b)



The product from part (a) (44 mg, 0.18 mmol) was added to a mixture of phosgene (0.24 ml of a 20% phosgene in toluene solution, 0.45 mol) in methylene chloride (1 ml) at 0° C. followed by the addition of triethylamine (25 μl, 0.18 mmol). The mixture was stirred at 0° C. for 1 hour. The reaction mixture was evaporated in vacuo. The residue was purified by flash chromatography (silica gel, 0–10% ethyl acetate/hexane) to give about 30 mg of the desired product as a colorless oil. IR(neat) 1732.0, 1694.5 cm^{-1}.

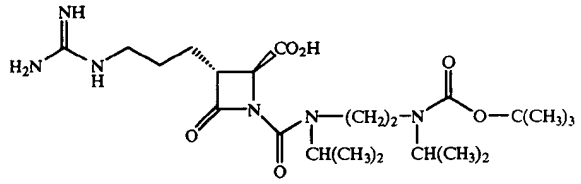
c)



Triethylamine (15 μl, 0.104 mmol) and dimethylaminopyridine (10–12 crystals) were added to a solution of the benzyl ester product from Example 1(c) (40 mg, 0.07 mmol) in methylene chloride (1 ml) followed by the addition of the chloro product from part (b) (25 mg, 0.084 mmol). The mixture was stirred for 48 hours and then evaporated in vacuo and purified by flash chromatography (silica gel, 0–30% ethyl acetate/hexane) to give 21 mg of the desired product as a colorless oil.

MS 843.5 $(M+H)^+$, 841.8 $(M-H)^-$; IR (film) 1785.1, 1733.1, 1681.7, 1640.9 cm^{-1}.

d)

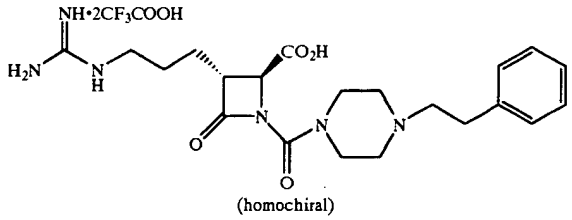


(homochiral)

10% Palladium on carbon catalyst (20 mg, wet type) was added to a solution of the product from part (c) (21 mg, 0.025 mmol) in 1,4-dioxane (5 ml) containing 1N HCl (25 μl, 0.025 mmol). Hydrogen gas was bubbled through the solution for 4 hours. The reaction mixture was filtered through a pad of Celite® which was then repeatedly washed with 1,4-dioxane (10 ml) and water (15 ml). The combined eluents were lyophilized. The white lyophilate was dissolved in water and passed through a plug of polyvinylpyrrolidone eluting with water. The eluents were lyophilized to

give 12 mg of the desired product as a white lyophilate. MS 485.3 $(M+H)^+$, 483.5 $(M-H)^-$. IR (KBr) 1778.0, 1665.0 cm^{-1}.

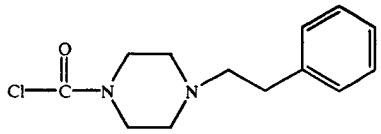
EXAMPLE 55



(homochiral)

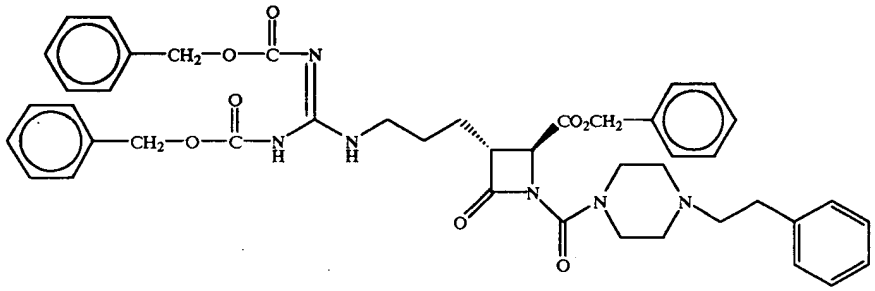
-continued

a)



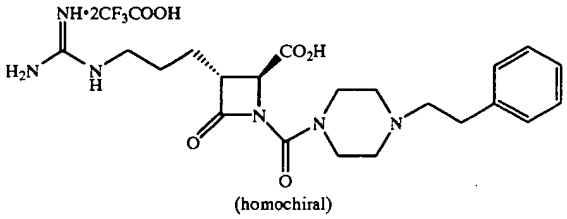
1-Phenethylpiperazine (0.5 g, 2.63 mmol) was added to a mixture of phosgene (3.5 ml of 20% phosgene in toluene solution, 6.6 mmol) in methylene chloride (2 ml) at 0° C., followed by the addition of triethylamine (0.37 ml, 2.63 mmol). The mixture was stirred at 0° C. for 2 hours and then evaporated in vacuo. The residue was suspended in ether, filtered and the eluents were condensed to give a cream solid. Purification by flash chromatography (silica gel, 0–10% ethyl acetate/hexane) gave 32.2 mg of the desired product as a crystalline, white solid. IR (film) 1729.3 cm^{-1}.

b)



Triethylamine (47 μl, 0.34 mmol) and dimethylaminopyridine (10–12 crystals) were added to a solution of the benzyl ester product from Example 1(c) (129 mg, 0.23 mmol) in methylene chloride (3 ml) followed by the addition of the chloro product from part (a) (86 mg, 0.34 mmol). The mixture was stirred at room temperature for 5 hours and was then evaporated in vacuo giving a pale yellow paste. Purification by flash chromatography (silica gel, 0–35% ethyl acetate/hexane) gave 120 mg of the desired product as a colorless oil. MS 789.4 $(M+H)^+$, 787.7 $(M-H)^-$; IR (film) 1785.5, 1732.1, 1679.1, 1639.4cm^{-1}.

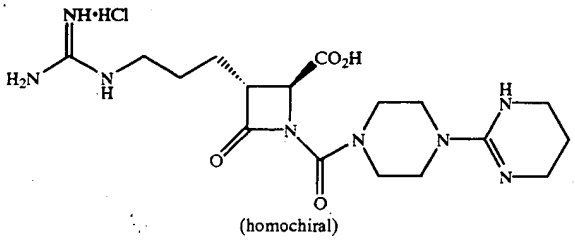
c)



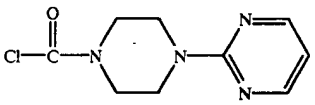
(homochiral)

10% Palladium on carbon catalyst (60 mg, wet added) was added to a solution of the product from part (b) (118 mg, 0.15 mmol) in 1,4-dioxane (8 ml) containing 1N HCl (0.18 ml, 0.18 mmol). Hydrogen gas was bubbled through the solution for 1 to 1.5 hours. The reaction mixture was filtered through a pad of Celite® which was repeatedly washed with 1,4-dioxane (10 ml) and water (15 ml). The combined eluents were lyophilized to obtain 77 mg of a white lyophilate. Purification by HPLC (reverse phase, methanol, water, trifluoroacetic acid) gave 52 mg of the desired product as a white lyophilate. MS 431.2 $(M+H)^+$, 429.3 $(M-H)^-$; IR (KBr) 1790.0, 1678.0 cm^{-1}.

EXAMPLE 56



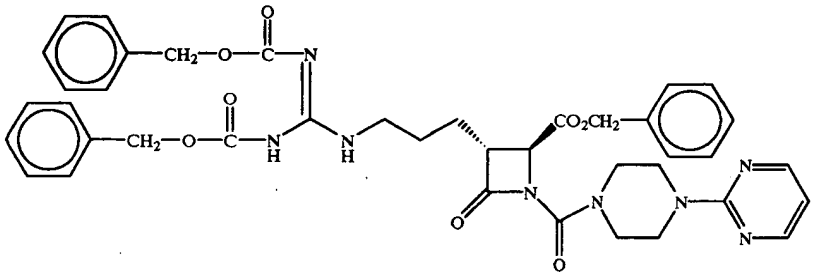
a)



A solution of 1-(2-pyrimidyl)piperazine dihydrochloride (4.0 g, 16.9 mmol) in 1N sodium hydroxide saturated with sodium chloride (40 ml) was extracted with ethyl acetate (2×30 ml). The organic layer was dried over sodium sulfate, filtered and concentrated to give 2.6 g of 1-(2-pyrimidyl) piperazine.

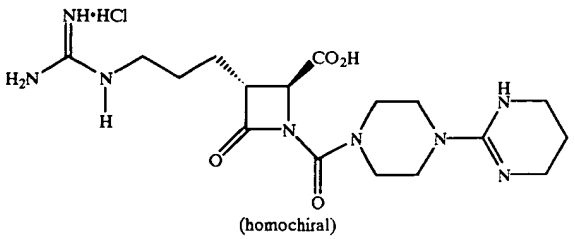
A solution of 1-(2-pyrimidyl)piperazine (2.6 g) in methylene chloride (5 ml) was added dropwise over 3 minutes to a solution of phosgene (25 ml, 20% in toluene, 47.3 mmol) in methylene chloride (15 ml) over solid sodium bicarbonate (3 g) under nitrogen at room temperature. The resulting solution was stirred vigorously for 10 minutes, filtered through a fritted funnel, and the remaining solids were washed with methylene chloride (2×5 ml). The combined eluent was concentrated under vacuum to give a white solid. The solid was then recrystallized from methylene chloride/hexane to give 3g of the desired product as a white solid. IR(film) 1735 cm^{-1}.

b)



The chloro product from part (a) (1.11 g, 4.8 mmol), triethylamine (700 μl, 5.0 mmol), dimethylaminopyridine (200 mg, 1.64 mmol) were added to a solution of the benzyl ester product from Example 1(c) (1.56 g, 3.23 mmol) in methylene chloride (15 ml) under nitrogen at room temperature. After stirring at room temperature for 7 hours, the reaction mixture was diluted with hexane (5 ml) and was then added to the top of a silica gel column (wetted with methylene chloride) for purification by flash chromatography (0 to 30% ethyl acetate/methylene chloride) to give 1.6 g of the derived product as a white foam. MS 763.2 $(M+H)^+$, 761.7 $(M-H)^-$; IR (KBr) 1788 cm^{-1}.

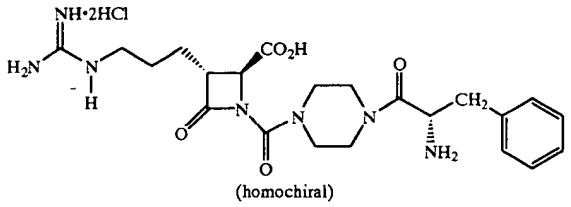
c)



The product from part (b) (1.6 g, 2.1 mmol) was deprotected and worked-up as described in Example 21(d) to give 854 mg of the desired product as white solid lyophilate. MS 409.2 $(M+H)^+$, 407.5 $(M-H)^-$; IR (KBr) 1777 cm^{-1}.

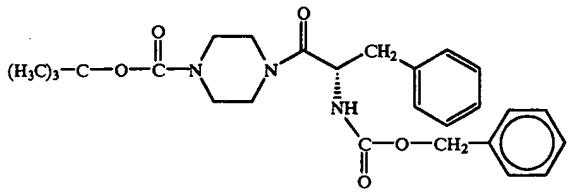
Anal. calc'd for $C_{17}H_{28}N_8O_4 \cdot 1.0$ HCl.1.54 H_2O: C, 43.20; H, 6.84; N, 23.71; O, 18.75; Cl, 7.50 Found: C, 43.31; H, 6.59; N, 23.09; O (not calculated); Cl, 7.06.

EXAMPLE 57



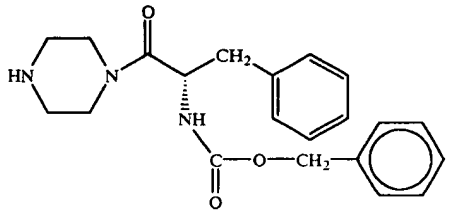
(homochiral)

a)



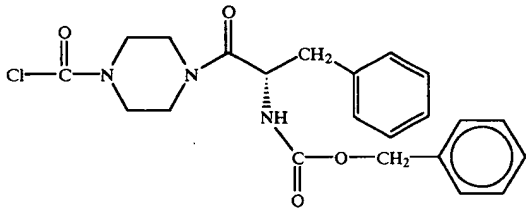
Triethylamine (0.22 ml, 1.6 mmol) and pyridine benzotriazol-1-yloxytris (dimethylamino)phosphonium hexafluorophosphate (0.83 g, 1.6 mmol) were added to a solution of N-carbobenzyloxy-L-phenylalanine (0.5 g, 1.6 mmol) in anhydrous methylene chloride (5 ml) followed by the addition of tert-butyl-1-piperazine carboxylate (0.3 g, 1.6 mmol). The mixture was stirred at room temperature for 2 hours. The mixture was then diluted with methylene chloride (30 ml) and washed with 1N HCl (1×25 ml), saturated sodium bicarbonate (1×25 ml), and saturated sodium chloride (1×25 ml). The organic phase was dried over sodium sulfate and concentrated to obtain a pale yellow oil. Purification by flash chromatography (silica gel, 0–20% ethyl acetate/hexanes) gave 554 mg of the desired product as a white foam. IR(film) 1698.2, 1649.6 cm^{-1}.

b)



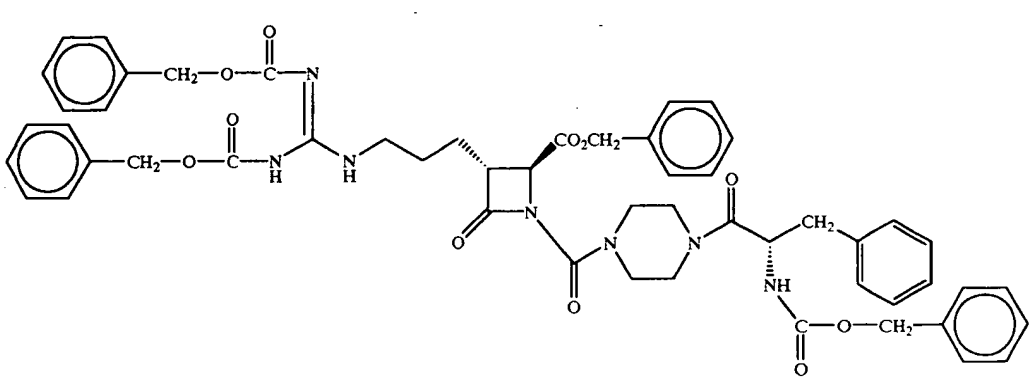
Trifluroacetic acid (3 ml) was added to a solution of the product from part (a) (550 mg, 1.14 mmol) in anhydrous methylene chloride (3 ml) at 0° C. The mixture was warmed to room temperature and stirred for 1.5 hours. The mixture was then condensed to give a colorless oil. The oil was dissolved in water, the pH was adjusted to 12–13 with sodium hydroxide (50% solution) and extracted with ethyl acetate (3×50 ml). The organic phase was dried over sodium sulfate and condensed to give 0.48 g of the desired product as a pale yellow oil. MS 368.2 $(M+H)^+$.

c)



The product from part (b) (0.322 g, 0.84 mmol) in methylene chloride (3 ml) was added to a mixture of phosgene (1.11 ml of a 20% phosgene in toluene solution, 2.1 mmol) in methylene chloride (3 ml) at 0° C., followed by the addition of triethylamine (0.12 ml, 0.84 mmol). The mixture was stirred at 0° C. for 1 hour. The reaction mixture was evaporated in vacuo. The residue was suspended in ether, filtered, and the eluents were concentrated to give a yellow oil. Purification by flash column chromatography (silica gel, 0–20% ethyl acetate/hexane) gave 0.243 g of the desired product as a colorless oil.

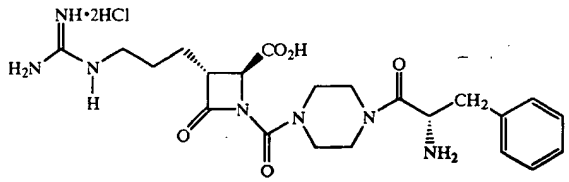
d)



Triethylamine (37 μl, 0.262 mmol) and dimethylaminopyridine (10–12 crystals) were added to a solution of the benzyl ester product from Example 1(c) (100 mg, 0.175-

mmol) in methylene chloride (3 ml), followed by the addition of the chloro product from part (c) (120 mg, 0.262 mmol). The mixture was stirred at room temperature for 4 hours and then evaporated in vacuo. Purification of the crude product by flash column chromatography (silica gel, 0–35%, ethyl acetate/hexane) gave 131 mg of the desired product as a colorless oil. MS 966.4 $(M+H)^+$, 964.6 $(M-H)^-$; IR (film) 1788.0, 1738.3, 1677.1, 1637.0 cm^{-1}.

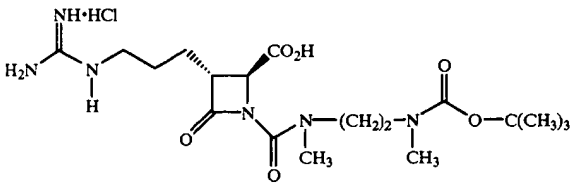
e)



(homochiral)

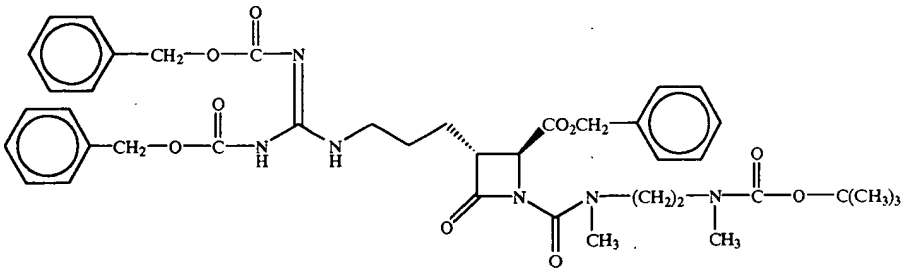
10% Palladium on carbon catalyst (65 mg, wet type) was added to a solution of the product from part (d) (127 mg, 0.132 mmol) in 1,4-dioxane (8 ml) containing 1N HCl (0.26 ml, 0.264 mmol). Hydrogen gas was bubbled through the solution for 1 to 1.5 hours. The reaction mixture was filtered through a pad of Celite® which was repeatedly washed with 1,4-dioxane (10 ml) and water (15 ml). The combined eluents were lyophilized to give 64 mg of the desired product as a pale yellow lyophilate. MS 474.2 $(M+H)^+$, 472.4 $(M-H)^-$; IR (KBr) 1786.0, 1730.0, 1647.0 cm^{-1}.

EXAMPLE 58



(homochiral)

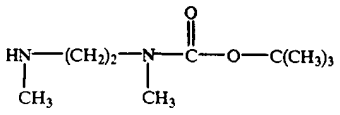
c)



Triethylamine (25 μl), dimethylaminopyridine (15 mg) and a solution of the chloro product from part (b) (45 mg, 0.18 mmol) in methylene chloride (1 ml) were added to a solution of the benzyl ester product from Example 1(c) (70 mg) in methylene chloride (2 ml). The mixture was stirred overnight at room temperature. The reaction was quenched with the addition of 1N potassium bisulfate (15 ml). The mixture was extracted with ethyl acetate (2×30 ml). The organic layers were combined and washed with brine (10

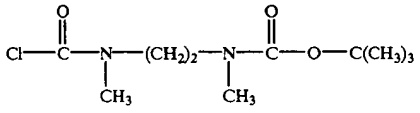
-continued

a)



A solution of di-tert-butyl dicarbonate (1.24 g, 5.67 mmol) and triethylamine (790 μl, 5.67 mmol) in tetrahydrofuran (15 ml) was added dropwise to a solution of N, N'-dimethylethylene diamine (500 mg, 5.67 mmol) in tetrahydrofuran (35 ml). The reaction mixture was stirred at room temperature for 4 days. The mixture was then filtered and the filtrate was concentrated to give the crude product as a colorless oil. Purification by flash chromatography (10% 2N ammonia in methanol/methylene chloride) provided 362 mg of the desired product as a colorless oil. IR(film) 1694 cm^{-1}.

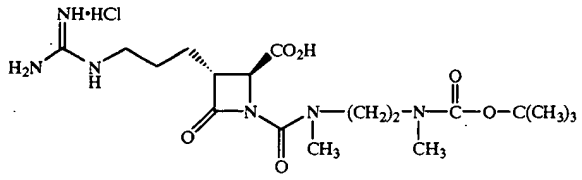
b)



A mixture of the product from part (a) (250 mg) and triethylamine (278 μl) in methylene chloride (3 ml) was added to a solution of phosgene in toluene (1.4 ml, 20%) at 0° C. The resultant mixture was stirred at 0° C. for 5 hours. Anhydrous ether (10 ml) was added and the solid was filtered off. The filtrate was evaporated to give the crude product as an orange oil which was purified by flash chromatography (20–30% ethyl acetate/hexane) to give 313 mg of the desired product as a colorless oil. IR (film) 1740 cm^{-1}, 1694 cm^{-1}.

ml), dried over magnesium sulfate and concentrated to give 101 mg of the crude product as a yellow oil. Purification using flash chromatography (30–50% ethyl acetate/hexane) gave 77 mg of the desired product as a colorless oil. IR (film) 1786 cm^{-1}, 1733 cm^{-1}, 1681 cm^{-1}, 1639 cm^{-1}.

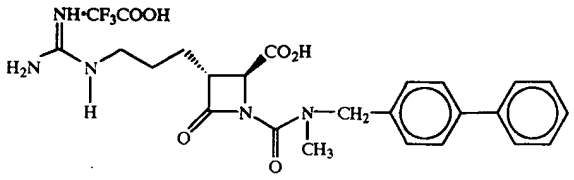
d)



(homochiral)

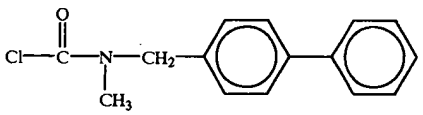
A mixture of the product from part (c) (74 mg, 0.094 mmol), 1N HCl (94 μl), and palladium on carbon catalyst (10%, 19 mg) in dioxane (2 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 1 hour. The reaction mixture was filtered through a Celite® cake and lyophilized to give 44 mg of the desired product as a white solid. MS 429.2 $(M+H)^+$, 427.5 $(M-H)^-$; IR (KBr) 1784, 1663 cm^{-1}.

EXAMPLE 59



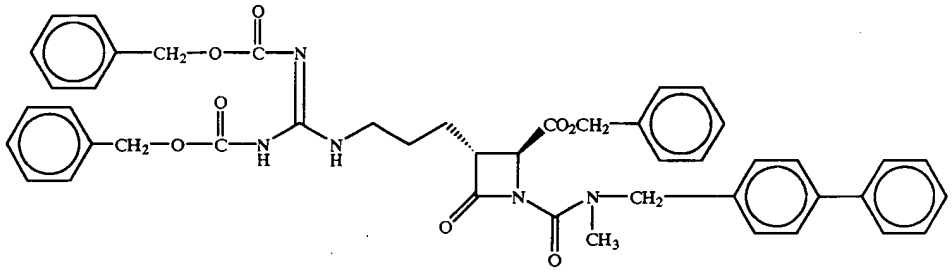
(homochiral)

a)



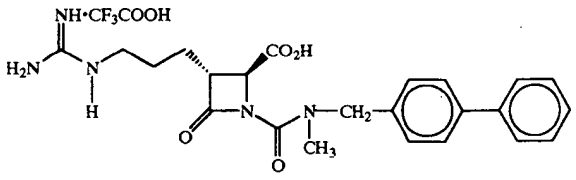
A solution of N-methyl-4-phenylbenzylamine [158 mg, 0.8 mmol, prepared as described by Dahn et al., Helv. Chim. Acta., 35, 1348–1358, (1952)] in toluene (3 ml) was added to a solution of phosgene (3 ml, 20% in toluene, 5.6 mmol) in toluene (3 ml) under nitrogen at room temperature followed by triethylamine (200 μl, 1.43 mmol). After stirring the reaction mixture at room temperature for 30 minutes, the solvents were removed under vacuum and the residue was purified by flash chromatography (silica gel, 100% methylene chloride) to give 136 mg of the desired product as an oily residue. IR(film) 1745 cm^{-1}.

b)



The chloro product from part (a) (100 mg, 0.38 mmol), triethylamine (53 μl, 0.38 mmol) and dimethylaminopyridine (10 mg, 0.08 mmol) were added to a solution of the benzyl ester product of Example 1(c) (145 mg, 0.25 mmol) in methylene chloride (3 ml) under nitrogen at room temperature. After stirring the reaction for 6 hours at room temperature, the reaction was diluted with hexane (1 ml) and added to the top of a silica gel column (wetted with hexane) for purification by flash chromatography (0 to 20% ethyl acetate in hexane) to give 148 mg of the desired product as a light brown wax. MS 796.5 $(M+H)^+$, 794.7 $(M-H)^-$; IR (film) 1786 cm^{-1}.

c)

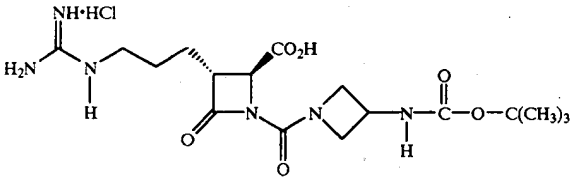


(homochiral)

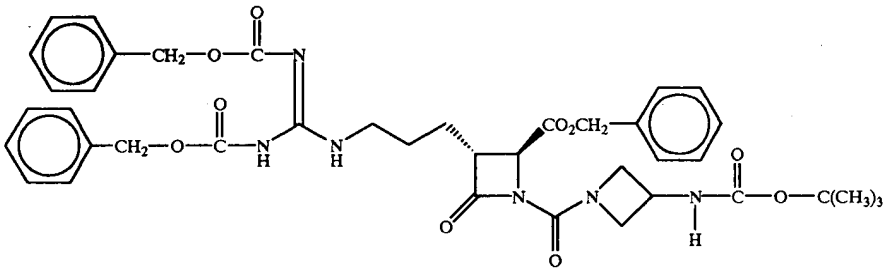
Deprotection and purification of the product from part (b) (148 mg, 0.186 mmol) according to the procedure of

Example 19(c) gave 33 mg of the desired product as a white foam. MS 438.2 $(M+H)^+$, 436.4 $(M-H)^-$; IR (KBr) 1788.0, 1699.0 cm^{-1}.

EXAMPLE 60

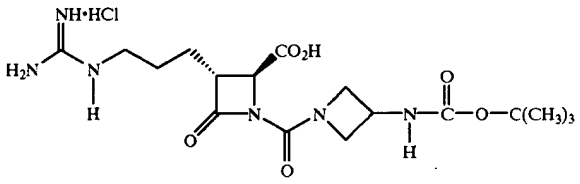


b)



The chloro product from part (a) (119 mg, 0.506 mmol) and the benzyl ester product from Example 1(c) (193 mg, 0.337 mmol) were dissolved in methylene chloride (2.5 ml). Triethylamine (71 μl, 0.506 mmol) was added followed by dimethylaminopyridine (8 mg, 0.067 mmole). After 12 hours the reaction mixture was concentrated and the crude product was purified by silica gel chromatography to give 180 mg of the desired product.

c)

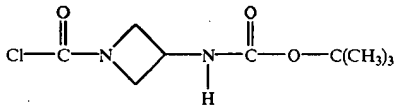


(homochiral)

The product from part (b) (80 mg, 0.104 mmol) was dissolved in 1,4-dioxane (1.0 ml) and water (0.10 ml). 1N HCl (104 μl, 0.104 mmol) was added followed by 10% palladium on carbon catalyst (16 mg). A hydrogen atmosphere was introduced via balloon. After 40 minutes of stirring at room temperature, the reaction mixture was diluted with water: 1,4-dioxane (1:1) and filtered. The filtrate was lyophilized to give 47 mg of the desired product. IR (KBr) 1792 cm^{-1}.

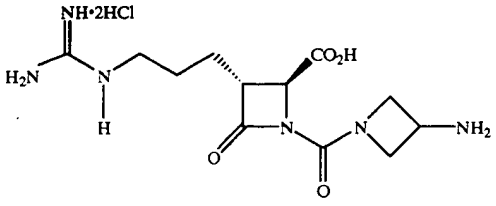
-continued

a)

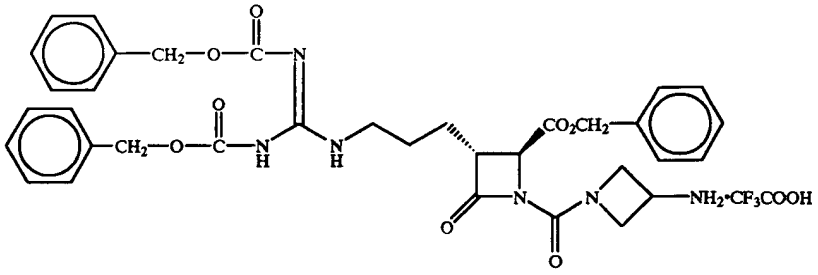


Phosgene (20% in toluene) (1.54 ml, 2.90 mmol) was added to a stirred solution of 3-tert-butoxycarbonylaminoazetidine [250 mg, 1.45 mmol, prepared as described by Arimoto et al., J. Antibiot., 39(9), 1243–56, (1986)] and triethylamine (222 μl, 1.6 mmol) in methylene chloride (5 ml) at 0° C. After 1 hour the reaction mixture was concentrated and the crude product was purified by silica gel chromatography to give 90 mg of the desired product.

EXAMPLE 61

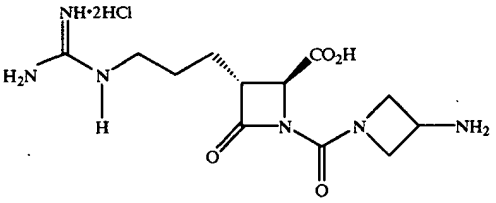


a) (homochiral)



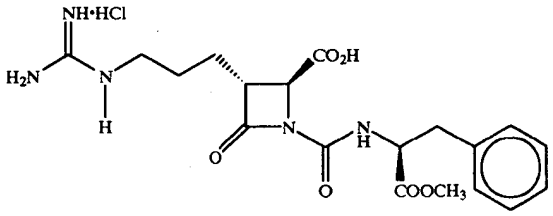
Trifluoroacetic acid (0.20 ml) was added drowise to a stirred solution of the product from Example 60 (b) (100 mg, 0.13 mmol) in methylene chloride at 0° C. The reaction mixture was then stirred at room temperature. After 40 minutes, the reaction mixture was concentrated in vacuo to give 120 mg of the desired product.

b)

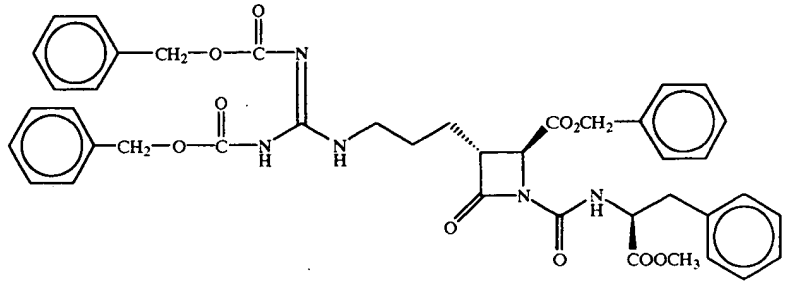


The product from part (a) (120 mg, 0.153 mmol) was deprotected and worked-up as described in Example 60(c) to give 51 mg of the desired product. IR(KBr) 1788 cm^{-1}.

EXAMPLE 62

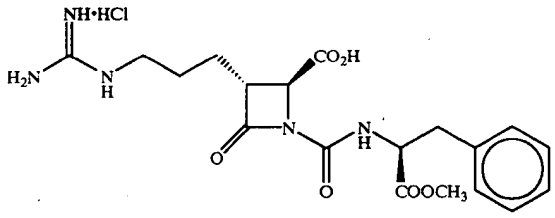


a)



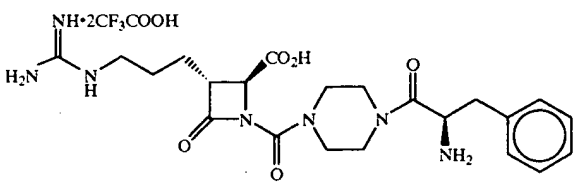
1N Sodium 1,1,1,3,3,3-hexamethyldilsilazane in tetrahydrofuran (143 μl, 0.14 mmol) was added dropwise over 5 minutes to a solution of the benzyl ester product from Example 1(c) (81.5 mg, 0.142 mmol) in dry tetrahydrofuran (5 ml) under nitrogen at −78° C. After warming to −20° C. and stirring for 30 minutes, methyl-(S)-(−)-2-isocyanato-3-phenylpropionate (29.2 mg, 0.14 mmol) dissolved in tetrahydrofuran (5 ml) was added dropwise. After one more hour of stirring, the reaction solution was allowed to warm to 0° C. and was then poured into potassium bisulfate solution (30 ml, pH adjusted to 3.5) containing crushed ice, followed by extraction with ethyl acetate (3×15 ml). The combined organic phase was washed with water and brine and finally dried over sodium sulfate. The filtrate was concentrated it vacuo to give 102 mg of crude product as a light yellow oil. Purification by flash chromatography on silica gel using ethyl acetate/hexane (1:1) as eluent gave 90.8 mg of the desired product as a colorless oil. IR(film) 1780 cm^{-1}, 1743 cm^{-1}, and 1639 cm^{-1}; MS 788.8 $(M+H)^+$.

b)



The product from part (a) (0.12 mmol) was dissolved in dioxane (5 ml). After addition of 10% palladium on carbon catalyst (40 mg) and 1N HCl in ether (120 μl), hydrogen was bubbled in the form of a constant slow stream over 90 minutes through the reaction suspension. After completion of the reaction as confirmed by TLC, a stream of nitrogen was used to remove excess hydrogen from the reaction material before filtering off the catalyst. Filtration through a layer of HyfloSuper Cel®, which was washed first with dioxane followed by dioxane/water yielded a clean filtrate. This was concentrated in vacuo and the remaining material lyophilized to give 45 mg of desired product as a white powder. IR (KBr) 1769 cm^{-1}, 1674 cm^{-1} and 1632 cm^{-1}; MS 420.1 $(M+H)^+$.

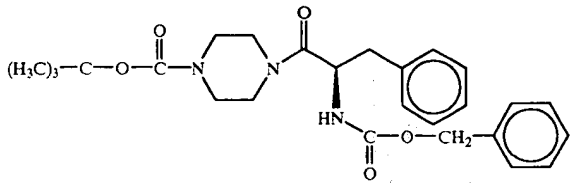
EXAMPLE 63



(homochiral)

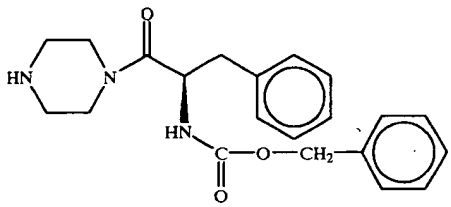
-continued

a)



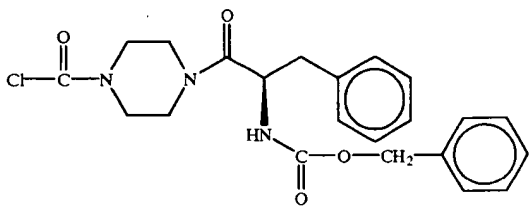
Triethylamine (0.37 ml, 2.68 mmol) and pyridine benzotriazol-1-loxytris(dimethylamino)phosphonium hexafluorophosphate (0.84 g, 2.68 mmol) were added to a solution of N-carbobenzyloxy-D-phenylalanine (0.84 g, 2.68 mmol) in anhydrous methylene chloride (10 ml), followed by the addition of tert-butyl-1-piperazine carboxylate (0.5 g, 2.68 mmol). After stirring the mixture for 5 hours at room temperature, methylene chloride (20 ml) was added and the mixture was washed with 1N HCl (1×25 ml), saturated sodium bicarbonate (1×25 ml), and saturated sodium chloride (1×25 ml). The organic phase was dried over sodium sulfate and condensed to give the crude product as a pale yellow oil. Purification by flash chromatography (silica gel, 0–30% ethyl acetate/hexane) gave 1.14 g of the desired product as a white foam.

b)



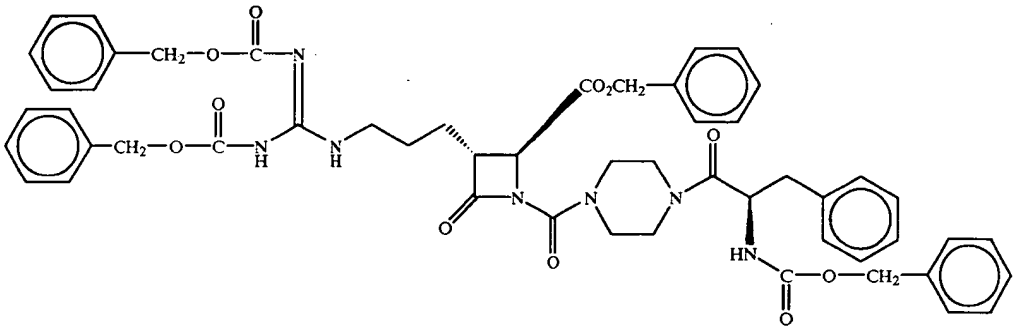
Trifluoroacetic acid (2 ml) was added to a solution of the product from part (a) (220 mg, 0.48 mmol) in anhydrous methylene chloride (2 ml) at 0° C. The mixture was warmed to room temperature and stirred for 1.5 hours. The mixture was condensed to give a colorless oil which was taken up in a solution of 1N HCl in ether (0.48 ml) and stirred vigorously. The resulting suspension was concentrated to give 230 mg of the hydrochloride salt of the desired product. MS 368.2 $(M+H)^+$.

c)



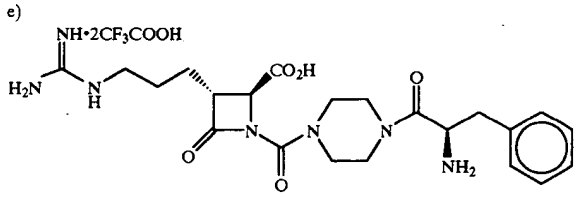
The product from part (b) (110 mg, 0.27 mmol) was added to a mixture of phosgene (0.36 ml of 20% phosgene in toluene solution, 0.68 mmol) and sodium bicarbonate (300 mg) in methylene chloride (4 ml). After stirring for 3 hours at room temperature, the mixture was filtered and the eluents were concentrated to give 165 mg of the desired product as a clear gel. IR (film) 1707.4, 1645.6 cm^{-1}.

d)



Triethylamine (31 μl, 0.23 mmol) and dimethylaminopyridine (10–12 crystals) were added to a solution of the benzyl ester product of Example 1(c) (86 mg, 0.15 mmol) in methylene chloride (3 ml), followed by the addition of the chloro product from part (c) (99 mg, 0.23 mmol). After stirring at room temperature for 1 hour, the mixture was concentrated and purified by flash column chromatography (silica gel, 0–50% ethyl acetate/hexane) to give 90 mg of the desired product as a colorless oil. MS 66.5 $(M+H)^+$, 964.7 $(M-H)^-$; IR(film) 1786.4, 1727.5, 1639.5 cm^{-1}.

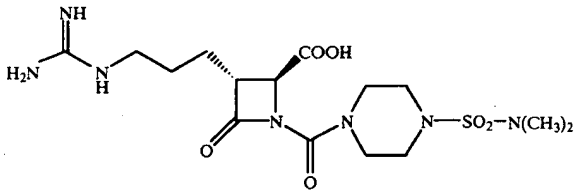
e)



(homochiral)

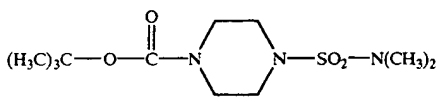
The product from part (d) (89 mg, 0.092 mmol) was deprotected and worked-up according to the procedure of Example 55(c) to give 68 mg of the desired product as a white lyophilate. MS 474.3 $(M+H)^+$, 472.6 $(M-H)^-$; IR (KBr) 1790.0, 1670.0 cm^{-1}.

EXAMPLE 64



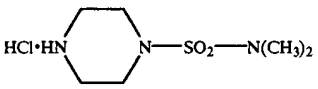
(homochiral)

a)



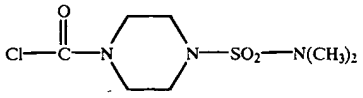
Diisopropylethylamine (560 μl), dimethylaminopyridine (33 mg) and dimethylsulfamoyl chloride (462 mg, 3.22 mmol) were added to a solution of N-(tert-butoxycarbonyl) piperizine (600 mg, 3.22 mmol) in methylene chloride (15 ml). The mixture was stirred overnight at room temperature. The reaction was quenched with the addition of 1N HCl solution (20 ml). The mixture was extracted with ethyl acetate (2×100 ml). The extracts were combined and washed with brine (2×20 ml), dried over magnesium sulfate and concentrated to give 0.93 g of the crude product as a white solid which was used without purification.

b)



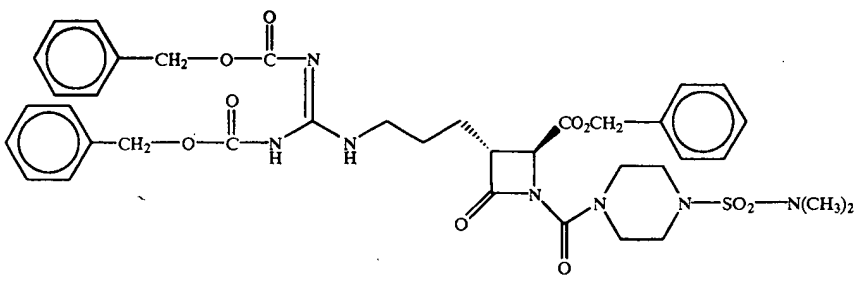
A mixture of the product from part (a) (0.60 g, 2.0 mmol), trifluoroacetic acid (15 ml) and methylene chloride (30 ml) was stirred at room temperature for 2 hours. TLC showed completion of the reaction. The solvent and excess trifluoroacetic acid were removed. The residue was dissolved in a minimum amount of methylene chloride followed by the addition of 1N HCl/ether (2.0 ml) and anhydrous ether (20 ml). The product was collected by filtration to give 430 mg of the desired product as a white powder. MS $(M+H)^+$ 194.1.

c)



Sodium bicarbonate (3.0 g) was added to a solution of phosgene (2.1 ml, 20% in toluene) in methylene chloride (20 ml) followed by the addition of the product from part (b) (300 mg, 1.3 mmol). The resultant reaction mixture was stirred at room temperature for 40 minutes. TLC showed completion of the reaction. The reaction was quenched by filtering off the sodium bicarbonate. The residue was evaporated to give 330 mg of the desired product. IR (film) 1738 cm^{-1}.

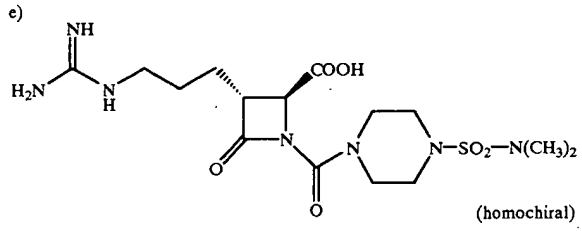
d)



Diisopropylethylamine (35 μl, 0.20 mmol), dimethylaminopyridine (23 mg) and a solution of the chloro product from part (c) (51 mg, 0.20 mmol) in methylene chloride (2 ml) were added to a solution of the benzyl ester product of Example 1(c) (100 mg) in methylene chloride (1 ml). The mixture was stirred at room temperature overnight. Analytical HPLC indicated the reaction was complete. The reaction was quenched with the addition of 1N potassium sulfate. The mixture was extracted with ethyl acetate. The extracts were combined and washed with brine, dried over magnesium sulfate, and concentrated. The resulting crude product was purified by flash chromatography (3% methanol/methylene chloride) to give 101 mg of the desired product as a white foam. MS $(M+H)^+$ 792.4, $(M-H)^-$ 790.7; IR (film) 1786 cm^{-1}, 1736 cm^{-1}, 1680 cm^{-1}, 1640 cm^{-1}.

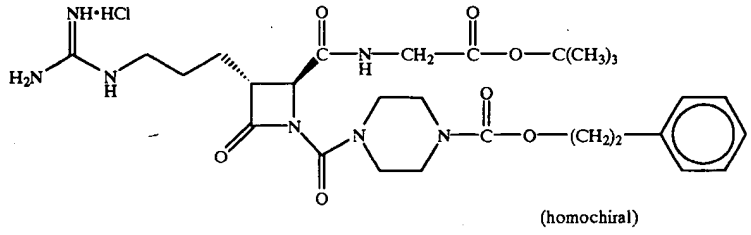
A mixture of the product from part (d) (95 mg, 0.12 mmol), 1N HCl (120 μl, 0.12 mmol), and 10% palladium on carbon catalyst (49 mg) in dioxane (3 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 2 hours. Analytical HPLC indicated completion of the reaction. The reaction mixture was filtered through a Celite® cake and concentrated to give the crude product (HCl salt). Purification by preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid) followed by passing through a polyvinylpyrrolidone column gave 32 mg of the desired product as a white fluffy powder. MS 434.3 $(M+H)^+$, 432.3 $(M-H)^-$; IR (KBr) 1778, 1663 cm^{-1}.

e)



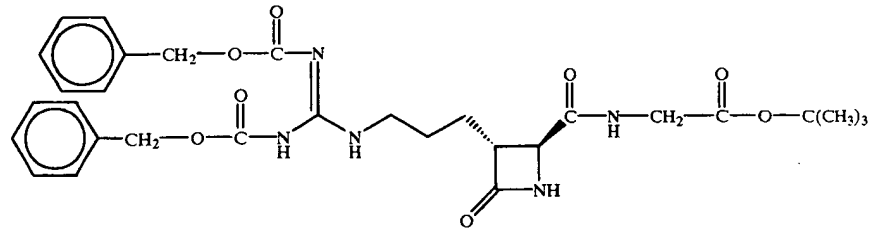
(homochiral)

EXAMPLE 65



(homochiral)

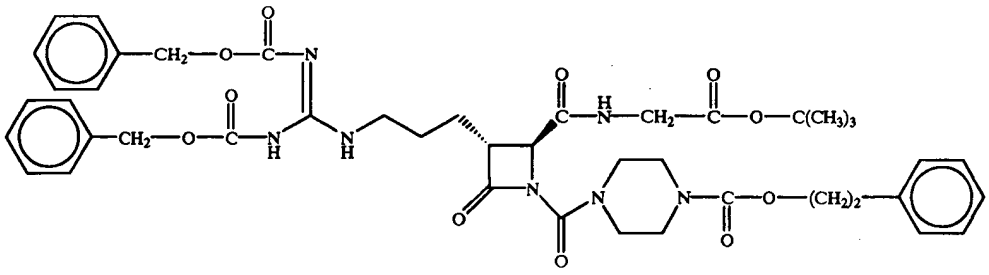
a)



A solution of the carboxylic acid azetidinone product of Example 1(b) (482 mg, 1.0 mmol) in tetrahydrofuran (5 ml) was cooled to −20° C. under an argon atmosphere and

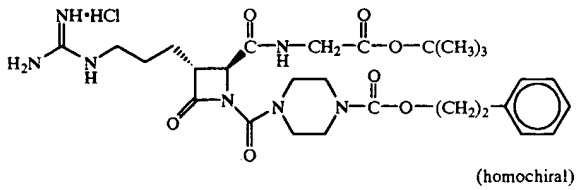
N-methylmorpholine (223 mg, 2.2 mmol) was added. 1.1 Equivalents of a 0.5 M solution of tert-butylglycine ester, hydrochloride (184 mg, 1.1 mmol) was added followed by the addition of benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (486 mg, 1.1 mmol). The reaction was stirred at −20° C. for 24 hours, poured into 5% potassium bisulfate solution and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine, and dried over sodium sulfate. The solvents were evaporated and the crude residue was purified by silica gel chromatography eluting with ethyl acetate to give 396 mg of the desired product as a colorless solid. MS 596 $(M+H)^+$.

b)



A solution of the product from part (a) (200 mg, 0.336 mmol) and triethylamine (38 mg, 0.37 mmol) in methylene chloride (4 ml) was stirred at room temperature and 1.1 equivalents of 1-phenethyloxypiperazine-4-carbonylchloride (110 mg, 0.37 mmol) was added. Dimethylaminopyridine (10 mg) was added and the reaction mixture was stirred for 30 hours. The reaction was diluted with methylene chloride, washed with brine, and dried over anhydrous sodium sulfate. The crude product was purified by flash chromatography on silica gel eluting with ethyl acetate yielding 210 mg of the desired product as a colorless glass-like residue. MS 856 $(M+H)^+$.

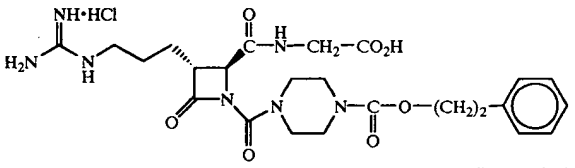
c)



(homochiral)

A solution of the product from part (b) (200 mg, 0.234 mmol) in dioxane (5 ml) containing 1.1 equivalents of HCl was stirred under a hydrogen atmosphere with 10% palladium on carbon catalyst (75 mg) for 2 hours. The reaction was filtered and the solvents lyophilized to yield 122 mg of the desired product as a colorless solid. MS 588 $(M+H)^+$.

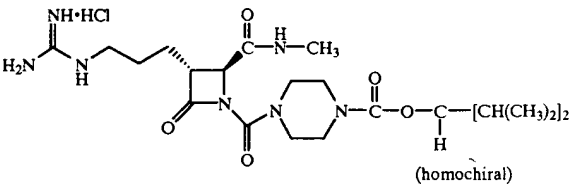
EXAMPLE 66



(homochiral)

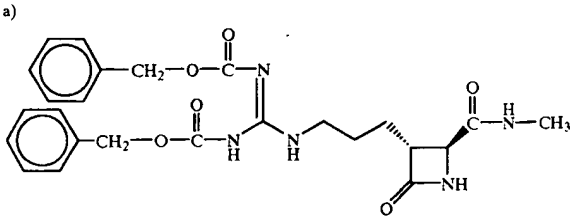
The product of Example 65 (30 mg, 0.05 mmol) was added to trifluoroacetic acid (1 ml) at 0° C. and the mixture was stirred for 30 minutes. The trifluoroacetic acid was evaporated and the residue was dissolved in water/dioxane (1:1) (1 ml) and lyophilized to give 22 mg of the desired product as a colorless solid. MS 532 $(M+H)^+$.

EXAMPLE 67



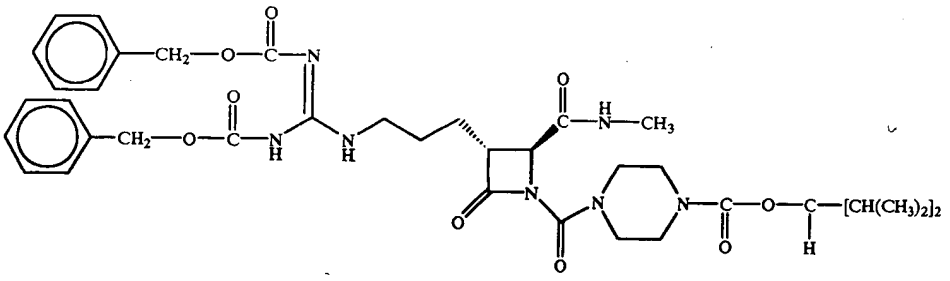
(homochiral)

a)



A solution of the carboxylic acid azetidinone product of Example 1(b) (150 mg, 0.311 mmol) in tetrahydrofuran (3 ml) was cooled to -20° C. under an argon atmosphere and N-methylmorpholine (34.6 mg, 0.342 mmol) was added. 1.1 Equivalents of a 2 M solution on monomethylamine in tetrahydrofuran was added followed by the addition of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (151 mg, 0.341 mmol). The reaction was stirred at -20° C. for 48 hours, poured into 5% potassium bisulfate solution, and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine, and dried over sodium sulfate. The solvents were evaporated and the crude residue was purified by silica chromatography eluting with ethyl acetate to give 124 mg of the desired product as a colorless solid. MS 496 $(M+H)^+$.

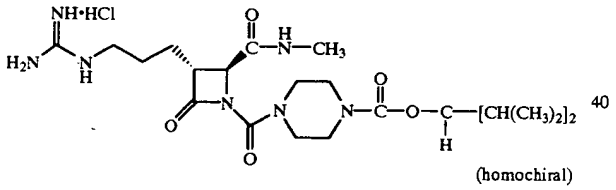
b)



A solution of the product from part (a) (100 mg, 0.2 mmol) and triethylamine (23 mg, 0.225 mmol) in methylene chloride (2 ml) was stirred at room temperature and 1.1 equivalents of 1-diisopropylmethoxy-carbonylpiperazine-4-carbonylchloride (65 mg, 0.225 mmol) was added. Dimethylaminopyridine (8 mg) was added and the reaction mixture was stirred for 16 hours. The reaction was diluted with methylene chloride, washed with brine, and dried over anhydrous sodium sulfate. The crude product was purified by flash chromatography on silica eluting with ethyl acetate to give 58 mg of the desired product as a colorless glass-like residue. MS 750 $(M+H)^+$.

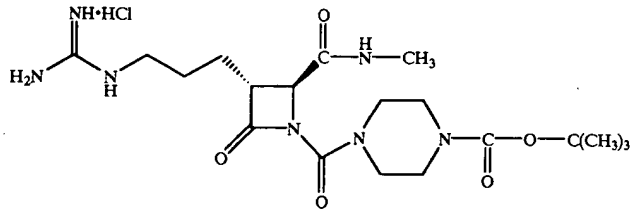
A solution of the product from part (b) (53 mg, 0.07 mmol) in dioxane (3 ml) containing 1.1 equivalents of 1N HCl was stirred under a hydrogen atmosphere with 10% palladium on carbon catalyst (20 mg) for 2 hours. The reaction was filtered and the solvents lyophilized to yield 32 mg of the desired product as a colorless solid. MS 482 $(M+H)^+$.

c)



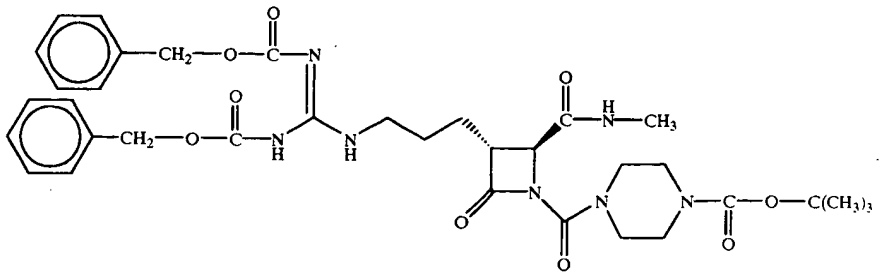
(homochiral)

EXAMPLE 68



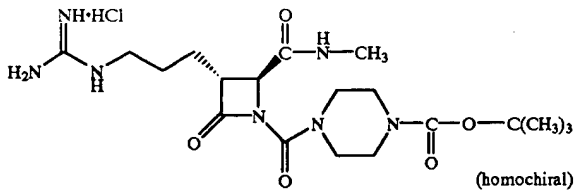
(homochiral)

a)



A solution of the product from Example 67(a) (120 mg, 0.242 mmol) and triethylamine (37 mg, 0.363 mmol) in methylene chloride (3.5 ml) was stirred at room temperature and 1.1 equivalents of 1-tert-butoxycarbonylpiperazine-4-carbonylchloride (90 mg, 0.363 mmol) was added. Dimethylaminopyridine (6 mg) was added and the reaction mixture was stirred for 2 hours. The reaction was diluted with methylene chloride, washed with brine, and dried over anhydrous sodium sulfate. The crude product was purified by flash chromatography on silica eluting with ethyl acetate to give 100 mg of the desired product as a colorless glass-like residue. MS 708 $(M+H)^+$.

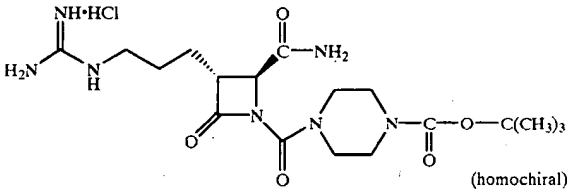
b)



(homochiral)

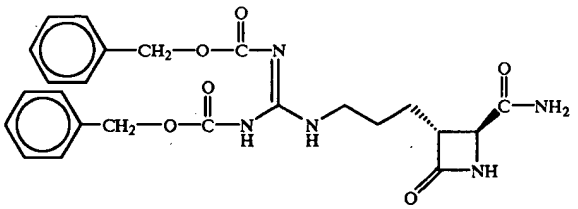
A solution of the product from part (a) (90 mg, 0.127 mmol) in dioxane (3 ml) containing 1.5 equivalents of HCl was stirred under a hydrogen atmosphere with 10% palladium on carbon catalyst (35 mg) for 2 hours. The reaction was filtered and the solvents lyophilized to give 38 mg of the desired product as a colorless solid. MS 439 $(M+H)^+$; $[\alpha]=14°$ (c=1, methanol).

EXAMPLE 69



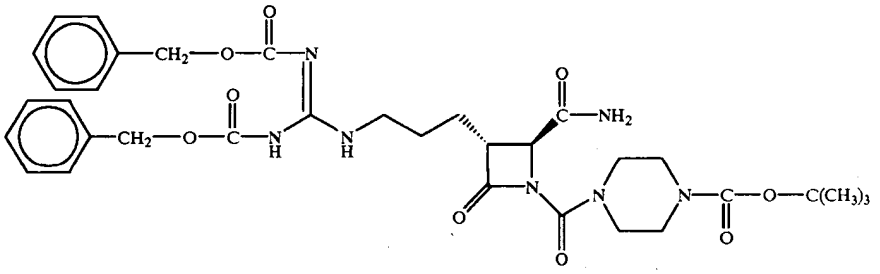
(homochiral)

a)



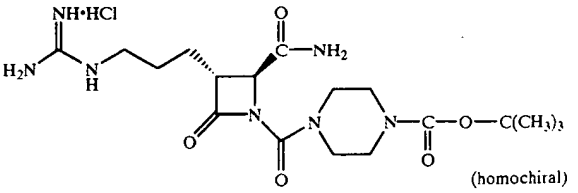
Following the procedure of Example 67(a) but substituting a 0.5 M solution of ammonia in dioxane for the 2M solution of monomethylamine, the desired product was obtained as a colorless solid. MS 482 $(M+H)^+$.

b)



Reacting the product from part (a) with 1-tert-butoxycarbonylpiperazine-4-carbonylchloride according to the procedure of Example 68(a), the desired product was obtained as a colorless glass-like residue. MS 694 $(M+H)^+$.

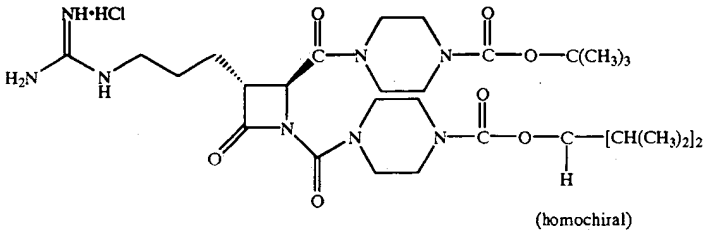
c)



(homochiral)

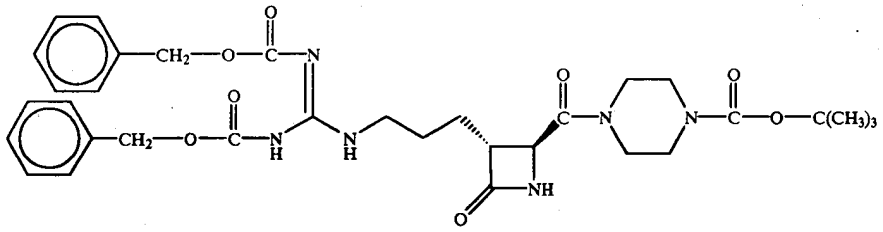
Deprotection and work-up of the product from part (b) according to the procedure of Example 68(b) gives the desired product as a colorless solid. MS 426 $(M+H)^+$.

EXAMPLE 70



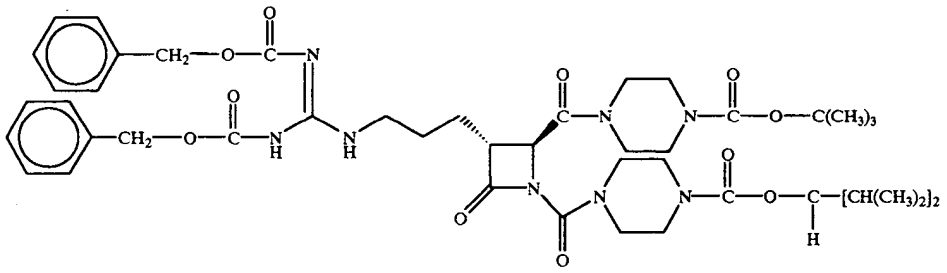
a)

(homochiral)



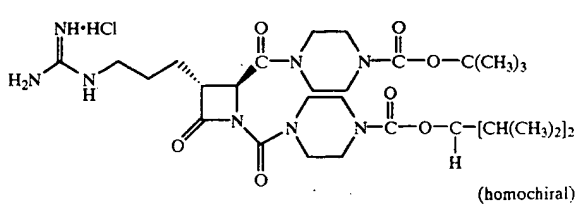
Following the procedure of Example 67(a) but substituting 1-tert-butoxycarbonylpiperazine for the monomethylamine, the desired product was obtained as a colorless solid. MS 651 $(M+H)^+$.

b)



A solution of the product from part (a) (100 mg, 0.2 mmol) and triethylamine (23 mg, 0.225 mmol) in methylene chloride (2 ml) was stirred at room temperature and 1.1 equivalents of 1-diisopropyl-methyloxycarbonylpiperazine-4-carbonylchloride (65 mg, 0.225 mmol) was added. Dimethylaminopyridine (8 mg) was added and the reaction mixture was stirred for 48 hours. The reaction was diluted with methylene chloride, washed with brine, and dried over anhydrous sodium sulfate. The crude product was purified by flash chromatography on silica eluting with ethyl acetate to give 68 mg of the desired product as a colorless glass-like residue. MS 906 $(M+H)^+$.

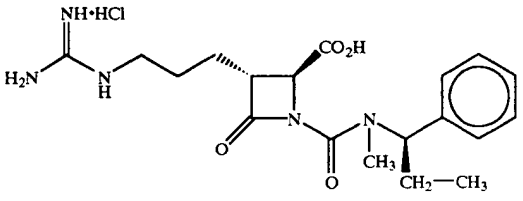
c)



(homochiral)

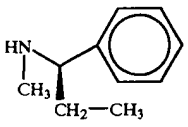
A solution of the product from part (b) (60 mg, 0.07 mmol) in dioxane (2 ml) containing 1.1 equivalents of 1.0 N HCl was stirred under a hydrogen atmosphere with 10% palladium on carbon catalyst (25 mg) for 2 hours. The reaction was filtered and the solvents lyophilized to yield 42 mg of the desired product as a colorless solid. MS 637 $(M+H)^+$.

EXAMPLE 71



(homochiral)

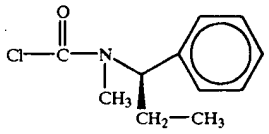
a)



Methyliodide (200 mg, 1.41 mmol) was added to a solution of (R)1-phenylaminopropane (420 mg, 2.82 mmol) and potassium carbonate (292 mg, 2.12 mmol) in tetrahydrofuran (5 ml). The reaction mixture was stirred at room temperature for 4 hours and then heated at 40–45° C. for 14 hours. The reaction mixture was filtered. The filtrate was concentrated and purified by flash column chromatography [elute with 5–10% ammonia (2M in methanol) in methylene

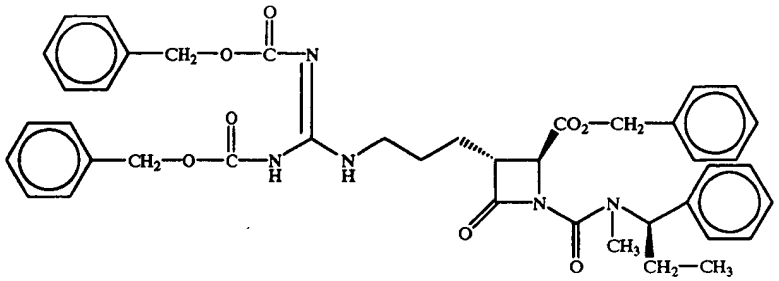
chloride] to yield 43 mg of the desired product as a light yellow oil.

b)



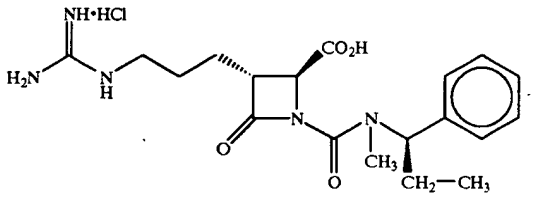
A solution of the product from part (a) (40 mg, 0.268 mmol) and triethylamine (27 mg, 0.268 mmol) in methylene chloride (1 ml) was added dropwise to a solution of phosgene (0.265 ml, 0.536 mmol, 20 % in toluene) in methylene chloride (1 ml) at 0° C. over 10 minutes. The reaction mixture was stirred at 0° C. for 1 hour. The reaction mixture was concentrated and the residue was added to anhydrous ether (20 ml). The formed white solid was filtered out. The resulting filtrate was concentrated to yield 51 mg of the desired product as a yellow oil.

c)



A mixture of the benzyl ester product from Example 1(c) (80 mg, 0.14 mmol), the chloro product from part (b) (44 mg, 0.21 mmol), dimethylaminopyridine (17.1 mg, 0.14 mmol) and triethylamine (21 mg, 0.21 mmol) in methylene chloride (3 ml) was stirred at room temperature for 7 hours. The reaction mixture was purified by flash column chromatography (eluting with 25% ethyl acetate in hexane) to yield 62 mg of the desired product as a colorless oil.

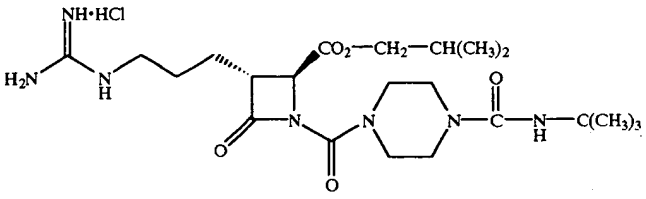
d)



(homochiral)

A mixture of the product from part (c) (60 mg, 0.08 mmol), 10% palladium on carbon catalyst (8.48 mg, 0.0008 mmol), and 1N HCl (0.08 ml, 0.08 mmol) in dioxane (3 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 1 hour. The reaction mixture was filtered through a Celite® cake. The resulting filtrate was lyophilized to yield 31 mg of the desired product as a white solid. MS 390.1 $(M+H)^+$; IR(film) 1780 cm^{-1}.

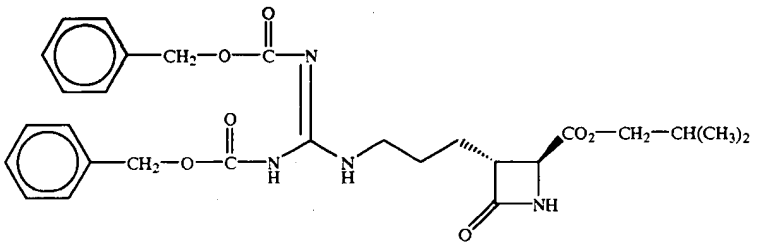
EXAMPLE 72



(homochiral)

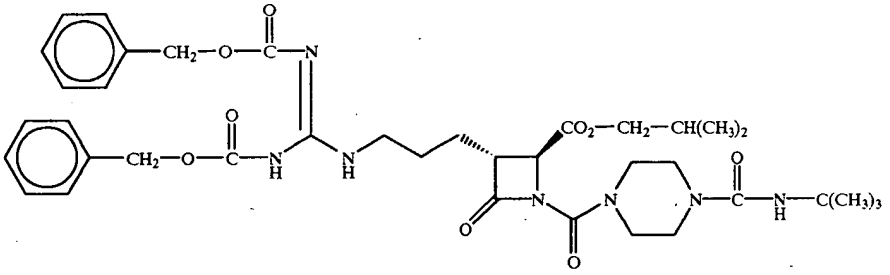
-continued

a)



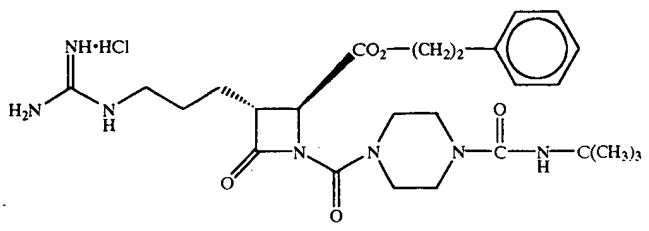
Cesium carbonate (29 mg, 0.088 mmol) was added to a stirred solution of the carboxylic acid azetidinone product of Example 1(b) (85 mg, 0.176 mmol) and 1-iodo-2-methylpropane (81 μl, 0.705 mmol) in dimethylformamide (500 μl) at room temperature. After 24 hours, the reaction was partitioned between ethyl acetate and water containing a small amount of sodium thiosulfate. The organic phase was isolated, washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography to afford 62 mg of the desired product.

b)



The product from part (a) (62 mg, 0.115 mmol) and the carbamoyl chloride product from Example 32 (c) were dissolved in methylene chloride (1.2 ml). Triethylamine (24 μl, 0.173 mmol) was added followed by dimethylaminopyridine (3 mg, 0.023 mmol). After 12 hours, the reaction mixture was concentrated and the crude product was purified by silica gel chromatography to give 65 mg of the desired product. After 30 minutes of stirring at room temperature, the reaction mixture was diluted with water: 1,4-dioxane (1:1, 4 ml) and filtered. The filtrate was lyophilized to afford 47 mg of the desired product. IR(KBr) 1788 cm^{-1}.

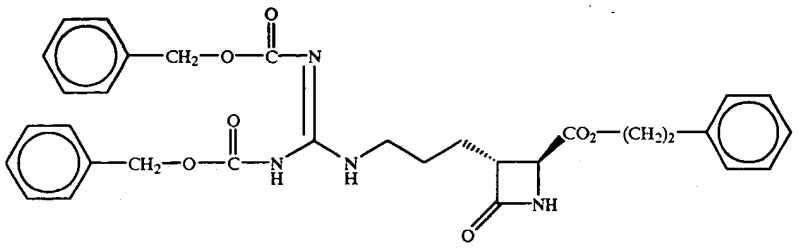
EXAMPLE 73



(homochiral)

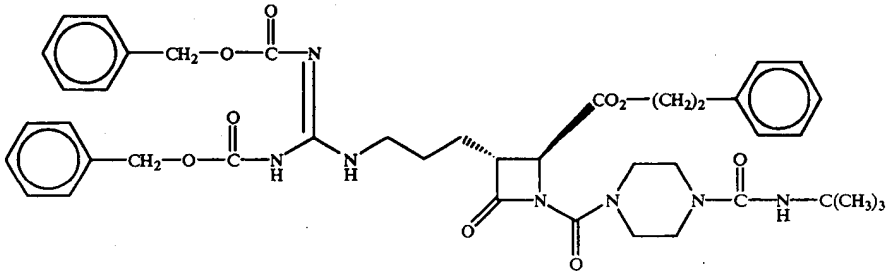
-continued

a)



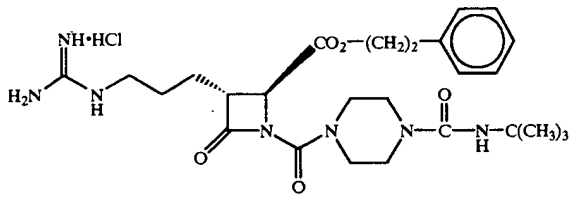
Following the procedure of Example 72 part (a) but substituting (2-iodoethyl)benzene for the 1-iodo-2-

b)



The product from part (a) (86 mg, 0.0147 mmol) and the carbamoyl chloride product from Example 32(c) (51 mg, 0.206 mmol) were reacted according to the procedure of Example 72 part (b) to give 98 mg of the desired product.

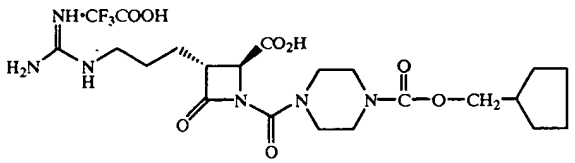
c)



(homochiral)

The product from part (b) (97 mg, 0.122 mmol) was deprotected and worked-up according to the procedure of methylpropane, the desired compound was obtained. Example 72(c) to give 67 mg of the desired product. IR (KBr) 1790 cm^{-1}.

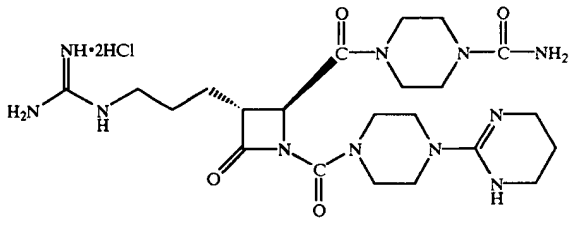
EXAMPLE 74



(homochiral)

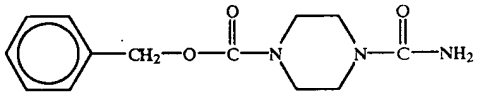
Following the procedure of Example 34 but employing yclopentylmethanol in place of the cyclohexylmethanol, the desired product was obtained as a colorless glass. MS 453.3 $(M+H)^+$, 451.4 $(M-H)^-$; IR(KBr) 1788 cm^-, 1665 cm^{-1}.

EXAMPLE 75



(homochiral)

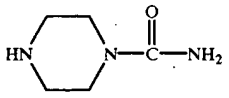
a)



Trimethylsilyl isocyanate (3.8 ml, 3.22 g, 28 mmol) was added dropwise over 15 minutes to a solution of N-carbobenzyloxypiperazine (5.5 g, 25 mmol) and diisopropylethylamine (9.6 ml, 7.1 g, 55 mmol) in tetrahydrofu-

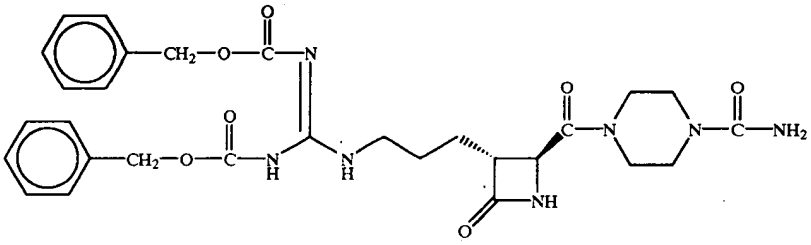
ran (100 ml) at room temperature under an argon atmosphere. The reaction was stirred overnight at room temperature. The reaction was poured into water and extracted with ethyl acetate, washed with water and brine, and dried over sodium sulfate. The crude product was purified by column chromatography eluting with 20% ethyl acetate/hexane to give 5.1 g of the desired product. MS $(M+H)^+$ 264.

b)



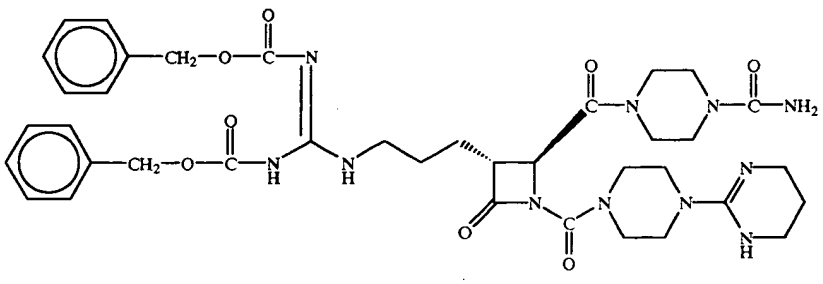
A mixture of the product from step (a) (3.96 g, 15 mmol) and palladium on carbon catalyst (10%, 2 g) in methanol (100 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 1.25 hours. Filtration and concentration of the reaction gave 2.0 of the desired product.

c)



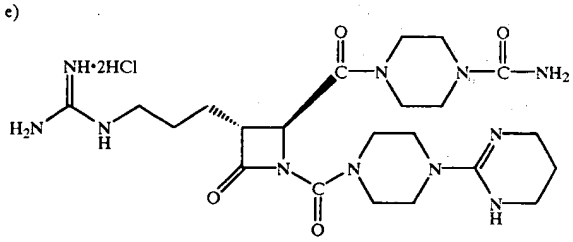
A mixture of the carboxylic acid azetidinone product of Example 1(b) (180 mg., 0.37 mmol), the product from step (b) (62 mg, 0.48 mmol), diisopropylethylamine (84 μl, 0.48 mmol), ethyl-3-(dimethylamino)propyl carbodiimide, hydrochloride salt (92 mg, 0.48 mmol) and 1-hydroxy-7-azabenzotriazole (65 mg, 0.48 mmol) in tetrahydrofuran (10 ml) was heated at 60° C. overnight. The mixture was diluted with methylene chloride, washed with water, dried over magnesium sulfate, and concentrated to give the crude product. Purification of the crude product by flash column chromatography (silica, 5–10% methanol/methylene chloride) gave 128 mg of the desired product as a white solid. MS 594.3 $(M+H)^+$, 592.3 $(M-H)^-$.

d)



A mixture of the product from step (c) (110 mg, 0.185 mmol), the chloro product from Example 56(a) (63 mg, 0.28 mmol), diisopropylethylamine (96 μl), and 4-dimethylaminopyridine (18 mg) in methylene chloride (4 ml) was stirred at room temperature for 18 hours. The reaction was quenched by the addition of saturated sodium chloride solution and extracted with ethyl acetate (3x50 ml). The extracts were combined, dried over magnesium sulfate, and concentrated. The crude product was purified by flash chromatography (0–15% methanol/methylene chloride) to give 114 mg of the desired product as a white solid. MS 784.5 $(M+H)^+$, 782.5 $(M-H)^-$; IR (KBr) 1782 cm^{-1}, 1732 cm^{-1}, 1643 cm^{-1}, 1586 cm^{-1}.

e)

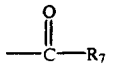


(homochiral)

A mixture of the product from step (d) (1.39 g, 1.77 mmol), 1N HCl (3.54 ml, 3.54 mmol) and palladium on carbon catalyst (10%, 750 mg) in dioxane (30 ml) was stirred under a hydrogen atmosphere (hydrogen balloon) at room temperature for 3.5 hours. Analytical HPLC indicated completion of the reaction. The reaction mixture was filtered through a Celite® cake and lyophilized to give 1.01 g as a white solid. MS 260.6 $(M+2H)^{2+}$; 1R 1780 cm^{-1}, 1632 cm^{-1}.

The following additional compounds of formula IV were also prepared:

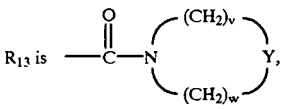
provided that

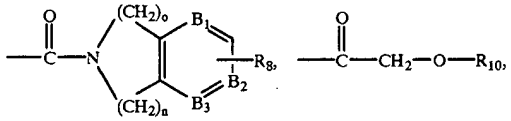


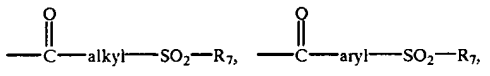
is other then alkylcarbonyl, phenylcarbonyl, substituted phenylcarbonyl, naphthylcarbonyl, substituted naphthylcarbonyl, phenylaminocarbonyl, substituted phenylaminocarbonyl, napthylaminocarbonyl, or substituted naphthylaminocarbonyl, or $—SO_2—R_7$ provided that $—SO_2R_7$ is other then alkylsulfonyl, phenylsulfonyl, substituted phenylsulfonyl, naphthylsulfonyl or substituted naphthylsulfonyl;

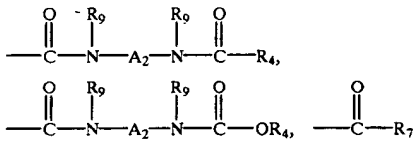
X_3 is phenylaminocarbonyl, substituted phenylaminocarbonyl, naphthylaminocarbonyl, substituted naphthylaminocarbonyl, alkylcarbonyl, phenylcarbonyl, substituted phenylcarbonyl, naphthylcarbonyl, substituted napthylcarbonyl, alkylsulfonyl, phenylsulfonyl, substituted phenylsulfonyl, naphthylsulfonyl, or substituted naphthylsulfonyl; and

R_{13} is

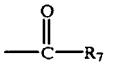




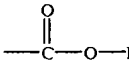




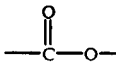
provided that



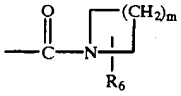
is other then phenylaminocarbonyl, substituted phenylaminocarbonyl, naphthylaminocarbonyl, substituted naphthylaminocarbonyl, carboxymethylaminocarbonyl, or alkoxycarbonylmethylaminocarbonyl, $—SO_2—R_7$ provided that $—SO_2R_7$ is other then alkylsulfonyl, phenylsulfonyl, substituted phenylsulfonyl, naphthylsulfonyl or substituted naphthylsulfonyl, or



provided that



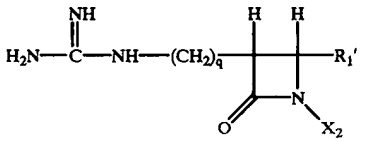
is other then alkoxycarbonyl, or



provided that if m is 1, 2 or 3 then R_6 is other then hydrogen, carboxy, alkoxycarbonyl or aryloxycarbonyl.

2. A compound of claim 1

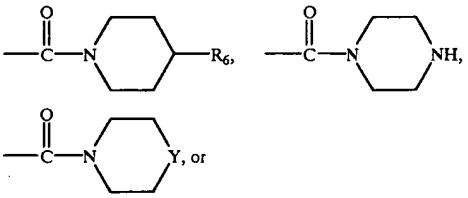
(IV)



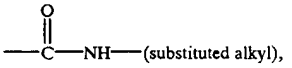
including an inner salt or a pharmaceutically acceptable salt thereof wherein:

q is 3;

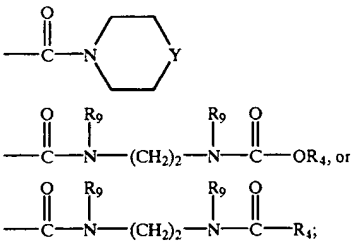
R_1' is carboxy,



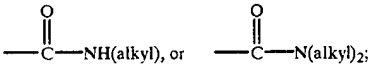
R_1' is carboxy,



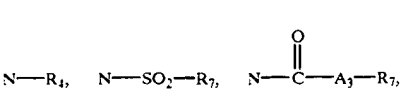
X_2 is

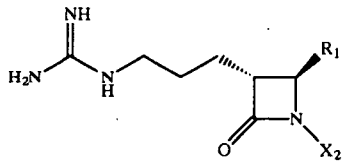


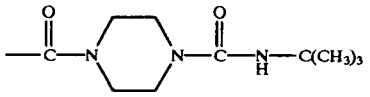
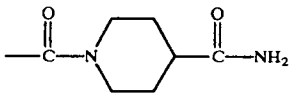
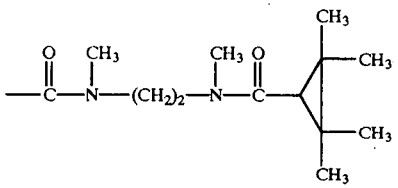
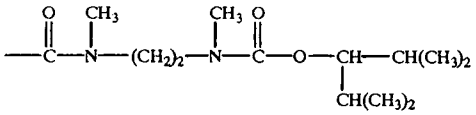
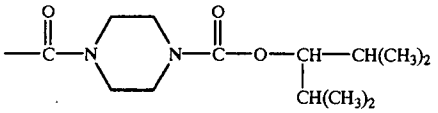
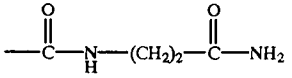
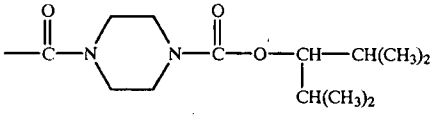
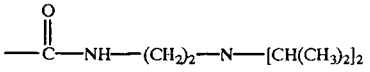
R_6 is aminocarbonyl,



Y is





Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
76			1.0 HCl	homochiral	536
77		$-CO_2H$	—	homochiral	453
78		$-CO_2H$	—	homochiral	471
79			1.0 HCl	homochiral	539
80			2.0 CF_3CO_2H	homochiral	595

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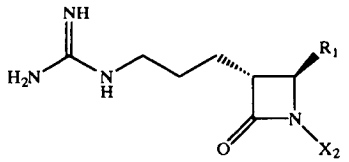
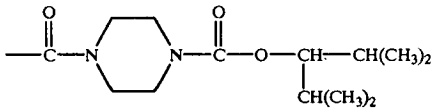
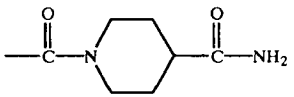
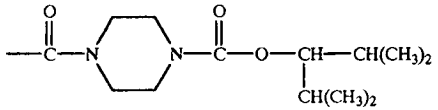
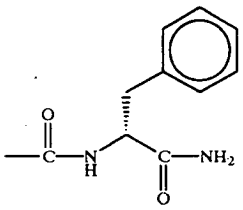
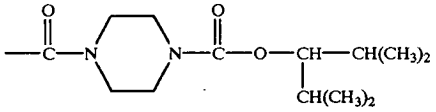
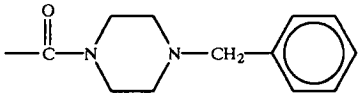
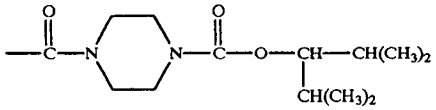
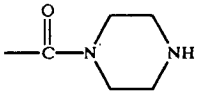
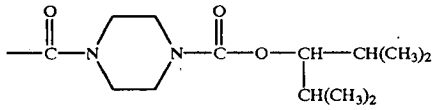
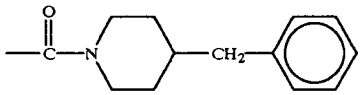


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
81			1.0 HCl	homochiral	579
82			1.0 HCl	homochiral	615
83			1.0 CF_3CO_2H	homochiral	627
84			2.0 CF_3CO_2H	homochiral	537
85			1.0 CF_3CO_2H	homochiral	626

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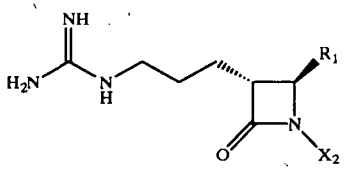
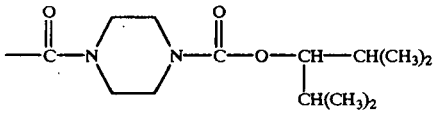
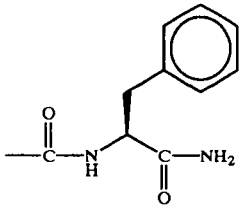
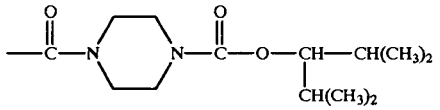
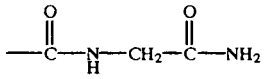
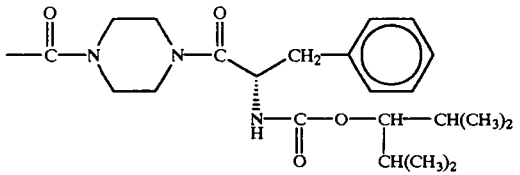
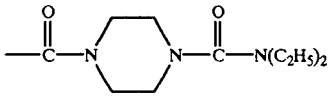
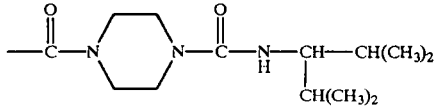


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
86			1.0 HCl	homochiral	615
87			1.0 HCl	homochiral	525
88		$-CO_2H$	—	homochiral	616
89		$-CO_2H$	—	homochiral	426
90		$-CO_2H$	—	homochiral	468

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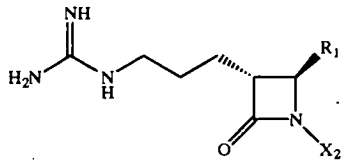
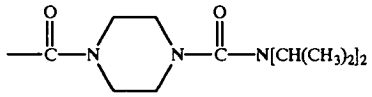
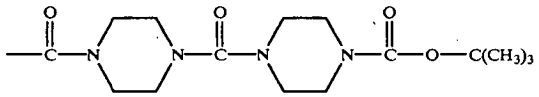
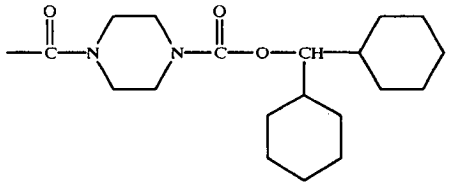
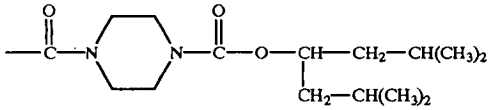
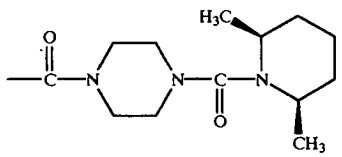


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
91		$-CO_2H$	—	homochiral	454
92		$-CO_2H$	—	homochiral	539
93		$-CO_2H$	1.0 HCl	homochiral	549
94		$-CO_2H$	1.0 CF_3CO_2H	homochiral	497
95		$-CO_2H$	—	homochiral	466

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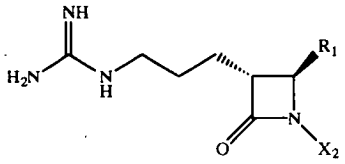
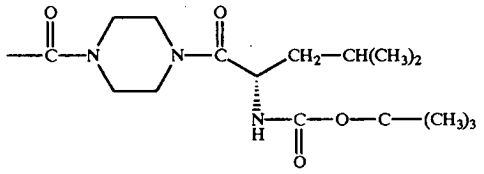
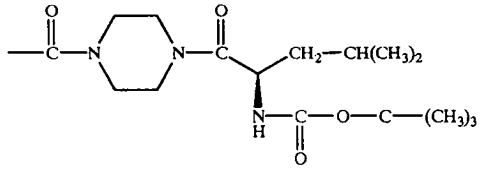
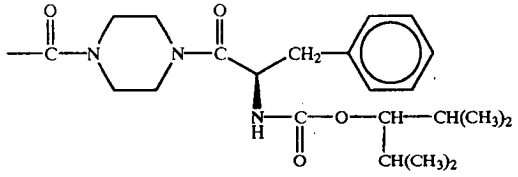
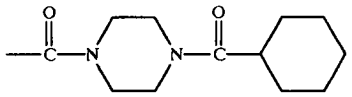
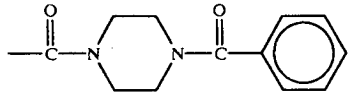


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
96		$-CO_2H$	—	homochiral	540
97		$-CO_2H$	—	homochiral	540
98		$-CO_2H$	—	homochiral	616
99		$-CO_2H$	1.0 HCl	homochiral	437
100		$-CO_2H$	1.0 HCl	homochiral	431

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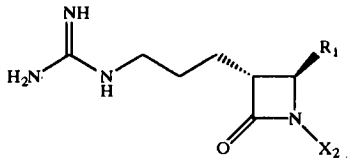
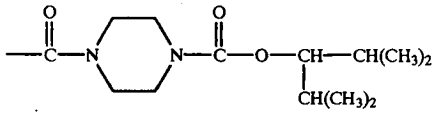
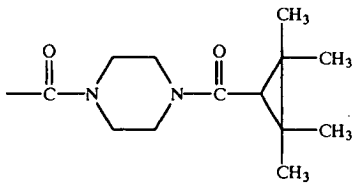
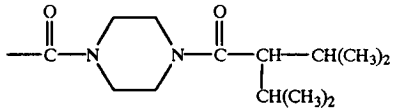
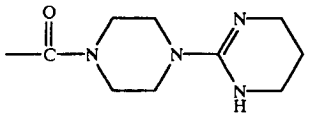
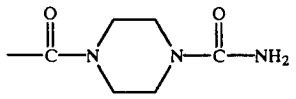


Ex	X_2	R_1	salt	stereochemistry	(M + H)+
101		—CO_2H	0.02 CF_3CO_2H	homochiral	469
102		—CO_2H	1.0 CF_3CO_2H	homochiral	451
103		—CO_2H	—	homochiral	453
104			2.0 HCl	homochiral	519

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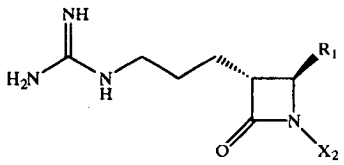
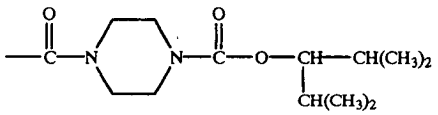
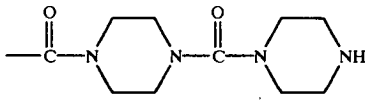
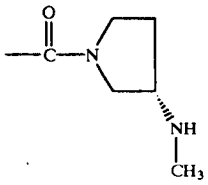
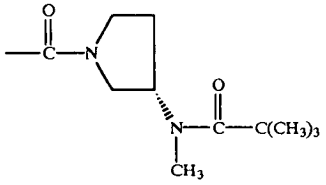
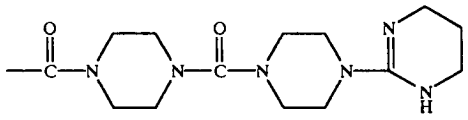
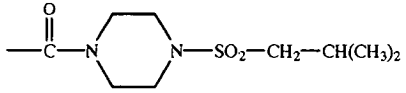


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
105			2.0 CF_3CO_2H	homochiral	649
106		$-CO_2H$	1.0 HCl	homochiral	341
107		$-CO_2H$	—	homochiral	441
108		$-CO_2H$	1.0 HCl	homochiral	521
109		$-CO_2H$	—	homochiral	447

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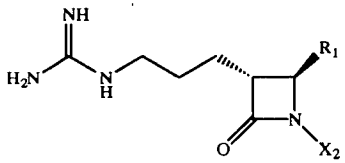
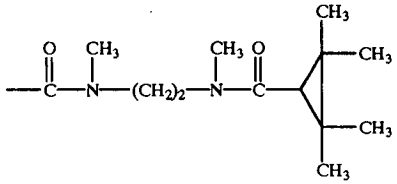
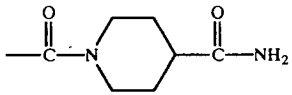
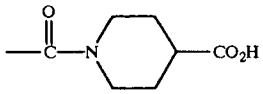
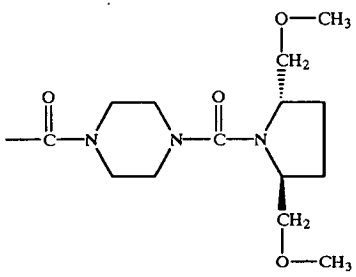


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
110			1.0 HCl	homochiral	563
111		$-CO_2H$	1.0 HCl	homochiral	370
112		$-CO_2H$	—	homochiral	512

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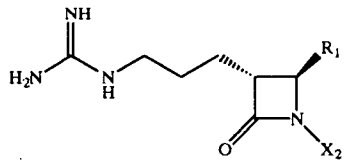
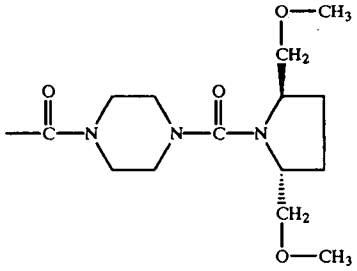
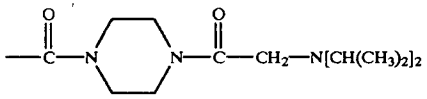
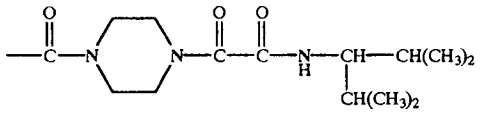
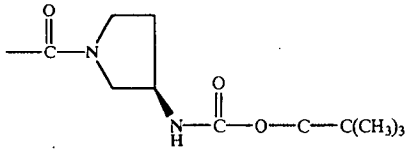
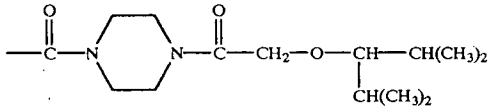


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
113		$-CO_2H$	—	homochiral	512
114		$-CO_2H$	2.0 CF_3CO_2H	homochiral	468
115		$-CO_2H$	—	homochiral	496
116		$-CO_2H$	—	homochiral	427
117		$-CO_2H$	—	homochiral	483

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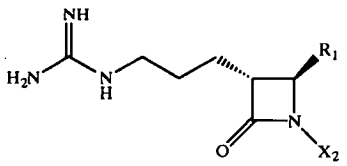
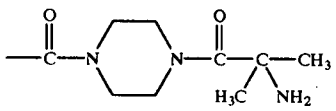
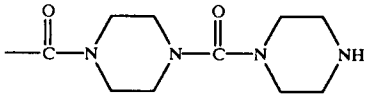
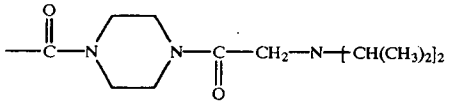
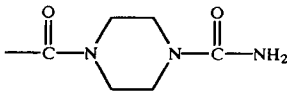
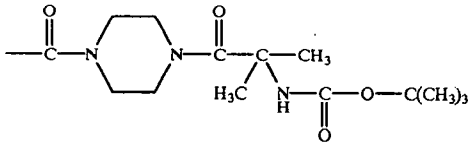
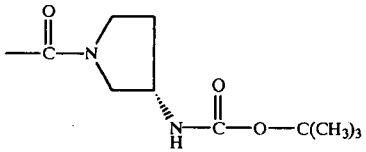
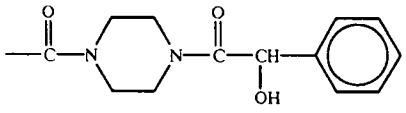


Ex	X_2	R_1	salt	stereochemistry	(M + H)+
118		$-CO_2H$	2.0 CF_3CO_2H	homochiral	434
119		$-CO_2H$	2.0 CF_3CO_2H	homochiral	439
120			2.0 CF_3CO_2H	homochiral	579
121		$-CO_2H$	—	homochiral	512
122		$-CO_2H$	—	homochiral	427
123		$-CO_2H$	1.0 HCl	mixture of homochiral diastereomers	461

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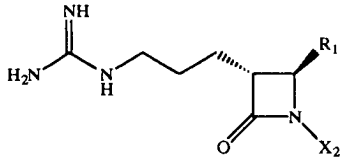
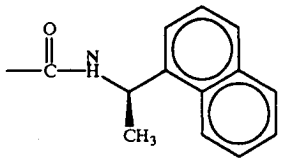
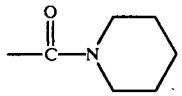
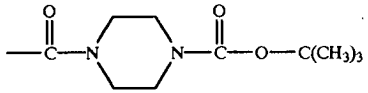
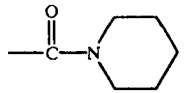
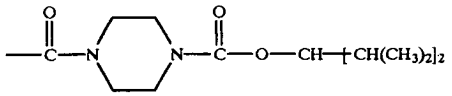
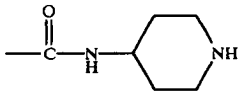
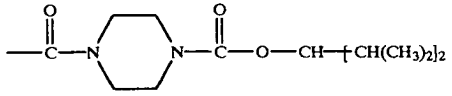
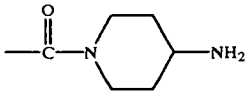


Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
124			1.0 HCl	homochiral	479
125			1.0 HCl	homochiral	494
126			2.0 CF_3COOH	homochiral	551
127			2.0 CF_3COOH	homochiral	594

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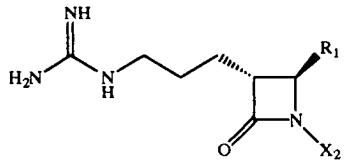
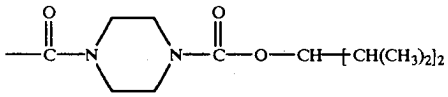
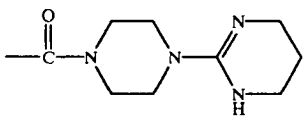
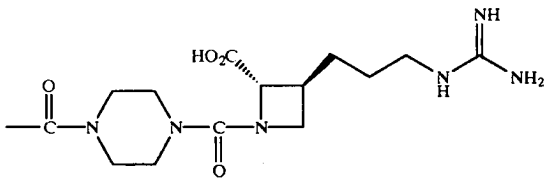
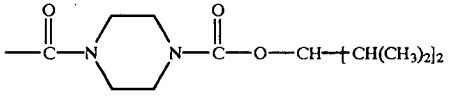
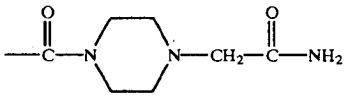
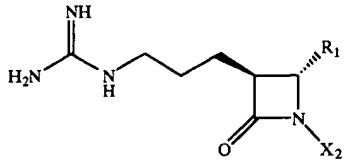
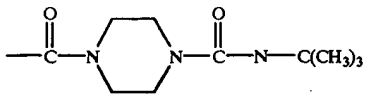
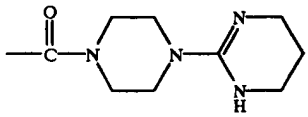
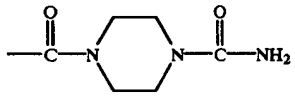
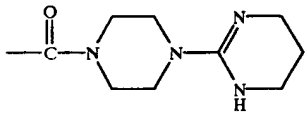
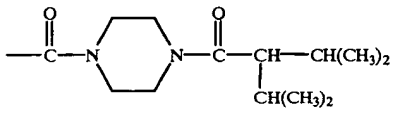
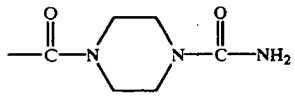


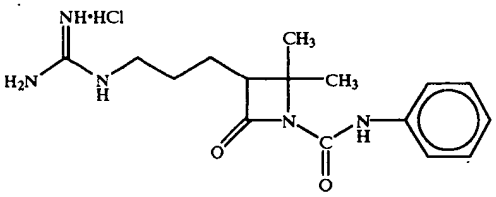
Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
128			2.0 CF_3COOH	homochiral	619
129		$-CO_2H$	2.0 CF_3COOH	homochiral	567
130			2.0 CF_3COOH	homochiral	594

The following additional compounds of formula IV were also prepared:



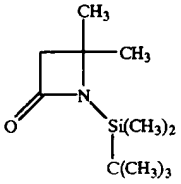
Ex	X_2	R_1	salt	stereochemistry	$(M + H)^+$
131		$-CO_2H$		homochiral	426
132			2.0 HCl	homochiral	520
133		$-CO_2H$	1.0 HCl	homochiral	409
134			1.0 HCl	homochiral	579

EXAMPLE 135



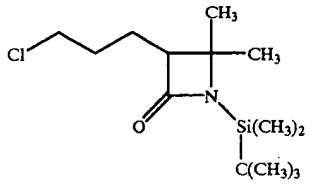
(racemate)

a)



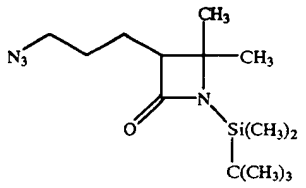
4,4-Dimethyl-2-azetidinone (17g, 0.171 mol) and tert-butyldimethylsilyl chloride (28.43 g, 0.188 mol) were dissolved in methylene chloride (270 ml). A solution of diisopropylethylamine (44.80 ml, 0.257 mol) in methylene chloride (130 ml) was added dropwise. The reaction mixture was stirred at room temperature for 24 hours and then concentrated. The residue was partitioned between ethyl acetate and water. The organic phase was washed with 1N potassium bisulfate, saturated sodium carbonate, saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography to give 34.55 g of the desired product.

b)



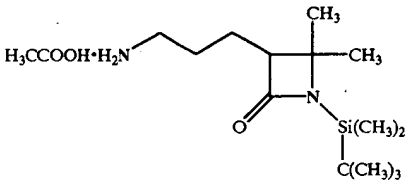
A 1.6 M hexane solution of n-butyl lithium (4.83 ml, 7.73 mmol) was added dropwise to a stirred solution of isopropylamine (1.08 ml, 7.73 mmol) in tetrahydrofuran (5.0 ml) at 0° C. After 30 minutes, the solution was cooled to −78° C. and a solution of the product from step (a) (1.50 g, 7.03 mmol) in tetrahydrofuran (2.5 ml) was added dropwise. After 40 minutes a solution of 1-chloro-3-iodopropane (1.72 g, 8.43 mmol) in tetrahydrofuran (2.5 ml) was added dropwise. The temperature was slowly raised to 0° C. After 1 hour the reaction mixture was quenched by the addition of 1N potassium bisulfate. The solution was partitioned between ethyl acetate and water. The organic phase was washed with 1N potassium bisulfate and saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography to give 1.46 g of the desired product.

c)



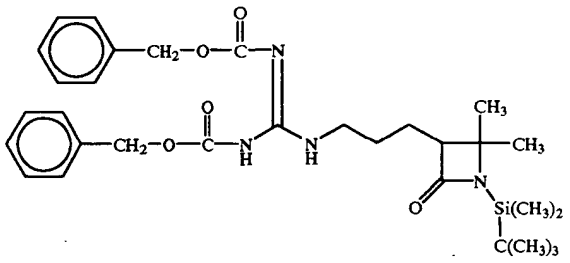
Sodium azide (0.91 g, 14.07 mmol) was added to a stirred solution of the product from step (b) (1.36 g, 4.69 mmol) and tetrabutylammonium iodide (0.35 g, 0.94 mmol) in dimethylformamide (10 ml). After 9 hours of heating at 45° C., the solution was cooled to room temperature and partitioned between ethyl acetate and water. The organic phase was washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated to afford 1.34 g of the desired product.

d)



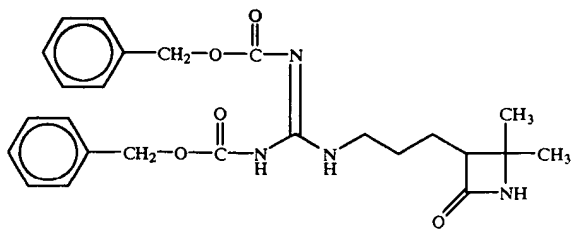
The product from step (c) (0.57g, 1.92 mmol) was dissolved in 1,4-dioxane. Acetic acid (0.11 ml, 1,92 mmol) was added followed by 10% palladium on carbon catalyst (0.15 mole%). A hydrogen atmosphere was introduced via balloon. After 1 hour of stirring at room temperature the solution was filtered and concentrated to give 0.59 g of the desired product.

e)



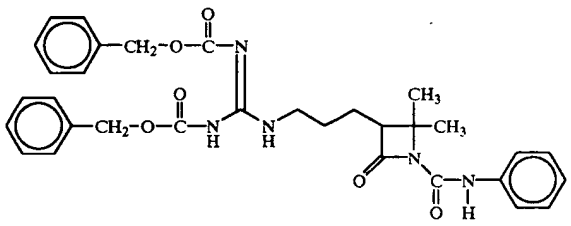
The product from step (d) (0.59 g, 1.78 mmol) was dissolved in acetonitrile (8.0 ml). Triethylamine (0.26 ml, 1.87 mmol) was added followed by N,N'-dicarbobenzyloxy—S-methylisothiourea (0.64 g, 1.78 mmol). After 12 hours of stirring at room temperature the solution was partitioned between ethyl acetate and water, the organic phase was washed with saturated sodium chloride, dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography to give 0.37 g of the desired product.

f)



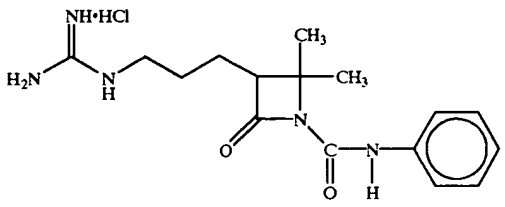
A 1.0 M tetrahydrofuran solution tetrabutylammonium fluoride (0.69 ml, 0.69 mmol) was added dropwise to a stirred soultion of the product from step (e) (0.36 g, 0.62 mmol) in tetrahydrofuran (5 ml) at 0° C. The reaction mixture was then stirred at room temperature. After 1 hour the solution was partitioned between ethyl acetate and water. The organic phase was washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography to give 276 mg of the desired product.

g)



A 1.0 M tetrahydrofuran solution of sodium bis (trimethylsilyl) amide (0.28 ml, 0.28 mmol) was added dropwise to a stirred solution of the product from step (f) (0.12 g, 0.26 mmol) in tetrahydrofuran (1.5 ml) at -78° C. After 30 minutes of stirring, phenyl isocyanate (42 μl, 0.39 mmol) was added dropwise. The temperature was slowly raised to 0° C. After 30 minutes the reaction mixture was quenched by the addition of a 4.0 pH buffer solution. The mixture was partitioned between ethyl acetate and water. The organic phase was washed with saturated sodium chloride, dried over magnesium sulfate and concentrated. The residue was purified by silica gel chromatography to give 0.16 g of the desired product.

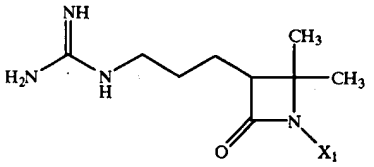
h)

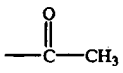
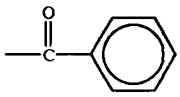
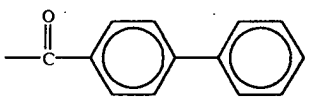
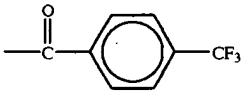
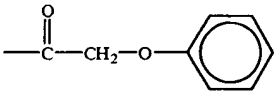
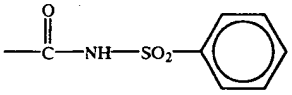
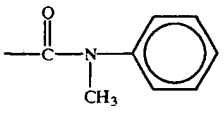
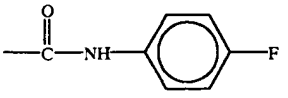
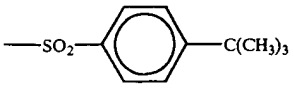
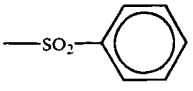


(racemate)

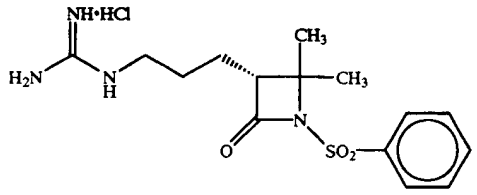
The product from part (g) (0.16 g, 0.27 mmol) was dissolved in 1,4-dioxane. 10% Palladium on carbon catalyst (0.15 mol %) was added followed by 4N HCl in 1,4-dioxane (68 μl, 0.27 mmol). A hydrogen atmosphere was introduced via balloon. After 30 minutes of stirring, water (0.20 ml) was added to keep the product in solution. After 30 more minutes, the reaction mixture was diluted with 50% acetonitrile in water (2.0 ml) and filtered. The solution was concentrated to remove organics and then lyophilized to give 84 mg of the desired product; IR (KBr) 1753, 1707 cm^{-1}; $(M+H)^+$=318.

The following additional compounds of formula II were also prepared



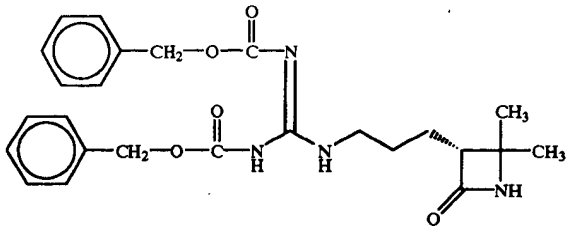
Ex	X_1	salt	stereochemistry	$(M + H)^+$
136		1.0 CF_3CO_2H	racemate	241
137		1.0 HCl	racemate	303
138		1.0 HCl	racemate	379
139		1.0 HCl	racemate	371
140		1.0 HCl	racemate	333
141		1.0 HCl	racemate	382
142		1.0 HCl	racemate	332
143		1.0 CF_3CO_2H	racemate	336
144		1.0 HCl	racemate	395
145		1.0 CF_3CO_2H	racemate	339

EXAMPLE 146



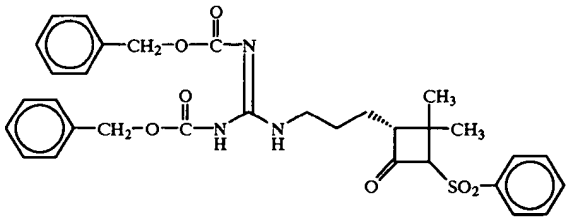
(homochiral)

a)



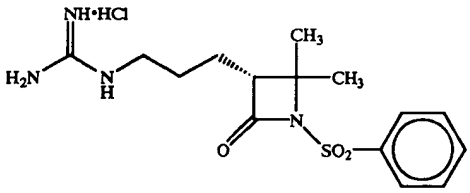
The racemic product from Example 135 step (f) was separated into pure enantiomers (−) isomer and (+) isomer on Chiralpak-AD prep-column eluting with 30% 2-propanol/hexane.

b)



A solution of the (+) isomer from step (a) (125 mg, 0.268 mmol) in tetrahydrofuran (2 ml) was cooled to −78° C. under an argon atmosphere. A 1M solution of sodium bis(trimethylsilyl)amide (0.536 ml) in tetrahydrofuran was added dropwise and the mixture was stirred for 20 minutes. Benzene sulfonylchloride (95 mg, 0.536 mmol) was added dropwise and the mixture was stirred at −78° C. for 4 hours. The reaction was diluted with aqueous 10% potassium bisulfate solution (10 ml) and extracted with ethyl acetate (3×10 ml). The combined organic phase was washed with water (25 ml) and brine (25 ml), and dried over sodium sulfate. The solution was filtered and the solvent evaporated to give an oil. The residue was purified by flash column chromatography (silica, ethyl acetate: hexane, 1:3) to give 149 mg of the desired product as a colorless oil; (M+H)+= 607.

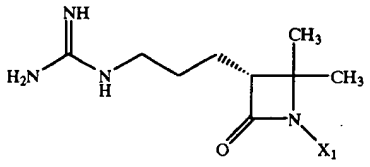
c)

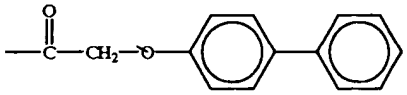
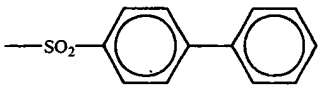


(homochiral)

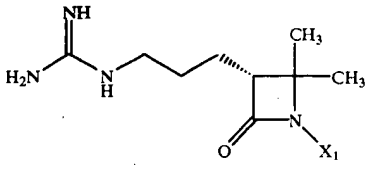
A solution of the product from step (b) (140 mg, 0.23 mmol) in dioxane (4 ml) containing aqueous 1N HCl (0.46 ml) and 10% palladium on carbon catalyst (70 mg) was stirred under a hydrogen atmoshpere for 1 hour. The reaction was filtered and lyophilized to give 78 mg of the desired product as a colorless solid; IR(KBr) 1778 cm^{-1}; (M+H)+= 339; $[\alpha]_D$=+12° (methanol, c=1).

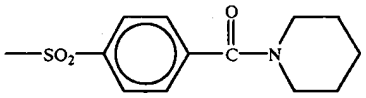
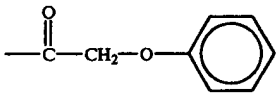
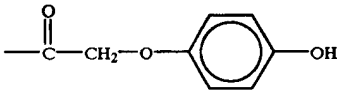
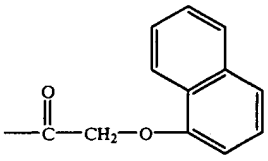
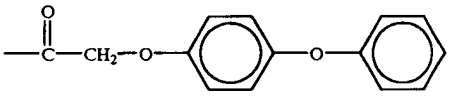
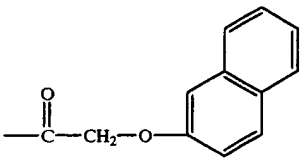
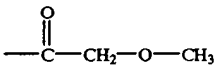
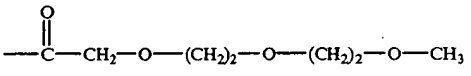
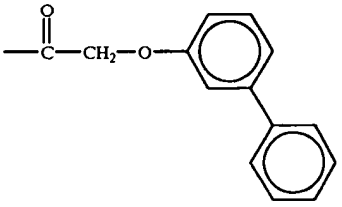
The following additional compounds of formula II were also prepared



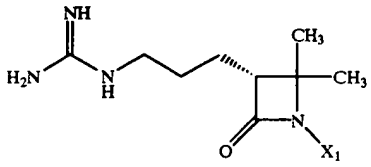
Ex	X_1	salt	stereochemistry	$(M + H)^+$
147		1.0 CF_3CO_2H	homochiral	409
148		1.0 HCl	homochiral	415

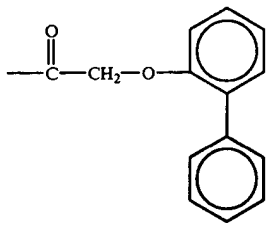
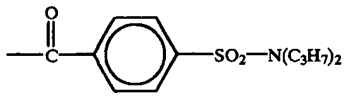
-continued



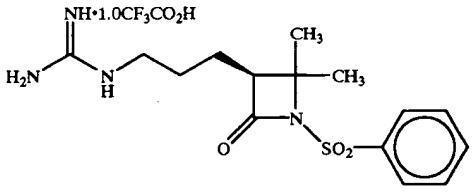
Ex	X_1	salt	stereochemistry	$(M + H)^+$
149		1.0 HCl	homochiral	450
150		1.0 HCl	homochiral	333
151		1.0 HCl	homochiral	349
152		1.0 HCl	homochiral	383
153		1.0 HCl	homochiral	425
154		—	homochiral	353
155		1.0 HCl	homochiral	270
156		1.0 HCl	homochiral	359
157		1.0 HCl	homochiral	409

-continued



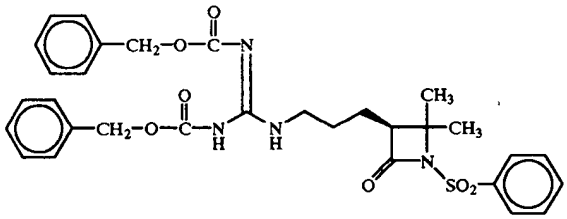
Ex	X_1	salt	stereochemistry	$(M + H)^+$
158		1.0 CF_3CO_2H	homochiral	409
159		1.0 HCl	homochiral	466

EXAMPLE 160



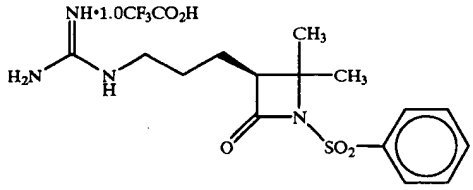
(homochiral)

a)



The (-) isomer from Example 146 step (a) (0.163, 0.349 mmol) was dissolved in tetrahydrofuran (2 ml) and cooled to -78° C. Sodium bis (trimethylsilyl) amide (0.52 ml, 0.524 mmol) was added dropwise. The mixture was stirred for 20 minutes. Benzenesulfonyl chloride (93 mg, 0.524 mmol) was added and the reaction mixture was stirred at -78° C. for 1.5 hours followed by stirring at room temperature overnight. The reaction was quenched with 0.5 N potassium bisulfate solution (25 ml) and extracted with ethyl acetate (2×20 ml). The organic phase was washed with brine (1×40 ml) and filtered over sodium sulfate. The filtrate was evaporated to a colorless oil. This was purified by reverse phase preparative HPLC (YMC ODS A 30×250 mm, 5 g column) to give 108 mg of the desired product as an oil; $(M+H)^+$= 607.2; $[\alpha]_D$=-7.0° (c=0.3, chloroform).

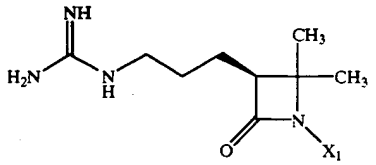
b)

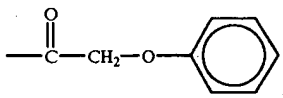


(homochiral)

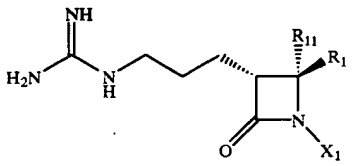
10% Palladium on carbon catalyst (50 mg) was added to a solution of the product from step (a) (105 mg, 0.173 mmol) in 1,4-dioxane (15 ml) containing 1N HCl (0.21 ml). Hydrogen gas was bubbled through the solution for 1 hour. The reaction mixture was filtered over a pad of Celite® and was washed repeatedly with 1,4-dioxane. The filtrate and washings were combined and evaporated to give a colorless oil (53 mg). This was further purified by reverse phase preparative HPLC (YMC ODS A 20×100 mm, 5 μl, fast elution column) to give 23 mg of the desired product as an oil; $(M+H)^+$=339; $[\alpha]_D$=-9.3° (c=0.42, methanol).

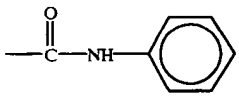
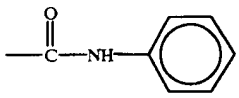
The following additional compounds of formula II were also prepared



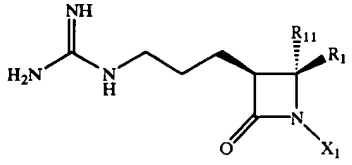
Ex	X_1	salt	stereochemistry	$(M + H)^+$
161		1.0 CF_3CO_2H	homochiral	333

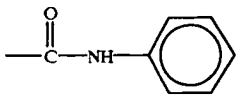
The following additional compounds of formula II were also prepared



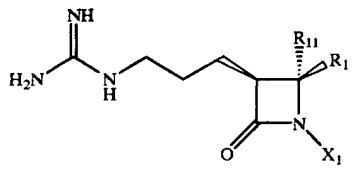
Ex	X_1	R_1	R_{11}	salt	stereochemistry	$(M + H)^+$
162		$—CO_2H$	CH_3	—	racemate	348
163		$—CO_2CH_3$	CH_3	1.0 HCl	racemate	362

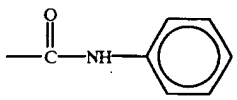
The following additional compounds of formula II were also prepared



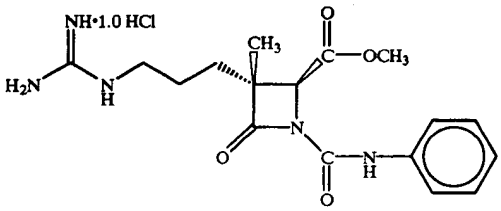
Ex	X_1	R_1	R_{11}	salt	stereochemistry	$(M + H)^+$
164		$—CO_2H$	CH_3	1.0 CF_3CO_2H	racemate	348

-continued



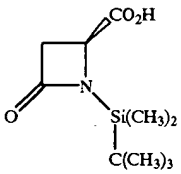
Ex	X_1	R_1	R_{11}	salt	stereochemistry	$(M + H)^+$
165		$-CO_2CH_3$	CH_3	1.0 HCl	racemate	362

EXAMPLE 166



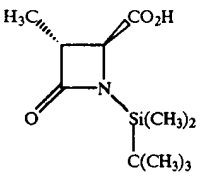
(racemate)

a)



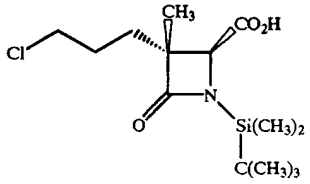
This racemic compound was prepared from D,L-aspartic acid following the procedure for the chiral compound from L-aspartic acid (P. E. Finke et al, J. Med. Chem., Vol. 38, p. 2449,1995).

b)



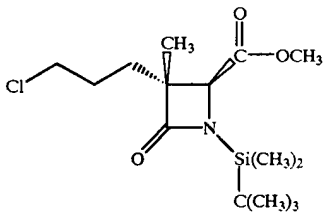
To diisopropylamine (5.6 ml, 40 mmol) in tetrahydrofuran (30 ml) at −20° C. under argon was added 2.5 M n-butyl lithium (14 ml) in hexane (35 mmol). The mixture was stirred for 15 minutes and cooled to −70° C. A solution of the racemic product from step (a) (4.00 g, 17.4 mmol) in tetrahydrofuran (16 ml) was added over 5 minutes and the reaction was warmed to −20° C. over 15 minutes. A solution of methyl iodide (2.72 ml, 43.7 mmol) in tetrahydrofuran (4 ml) was added and the reaction was stirred between −20° C. and 0° C. for 30 minutes. Dry ethyl ether (50 ml) was added and the reaction was poured into a mixture of ice and 80 ml. of 1N HCl. The layers were separated and the aqueous layer was extracted twice with brine, dried over sodium sulfate and concentrated to an amorphous solid. Treatment with hexane and ethyl acetate gave 2.86 g of the desired product as crystalline material.

c)



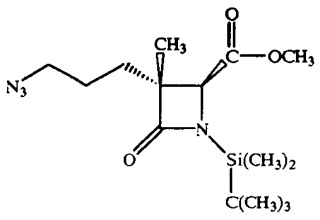
To diisopropylamine (3.62 ml, 25.8 mmol) in tetrahydrofuran (20 ml) at −20° C. under argon was added 2.5 M n-butyl lithium (9.2 ml) in hexane (23 mmol). The mixture was stirred for 15 minutes and cooled to −70° C. A solution of the product from step (b) (2.76 g, 11.3 mmol) in tetrahydrofuran (12 ml) was added over 5 minutes and the reaction was warmed to −20° C. over 15 minutes. A solution of 1-chloro-3-iodopropane (2.5 ml, 23.3 mmol) in tetrahydrofuran (5 ml) was added and the reaction was stirred between −20° C. and 0° C. for 30 minutes. Dry ethyl ether (50 ml) was added and the reaction was poured into a mixture of ice and 52 ml of 1N HCl. The layers were separated and the aqueous layer was twice extracted with ethyl ether. The combined extracts were washed twice with brine, dried over sodium sulfate and concentrated to an oil. Repeated concentration of the oil from ethyl ether and hexane gave 3.35 g of the desired product as an oil.

d)



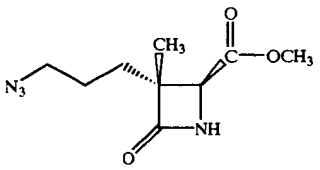
The product from step (c) (1.92 g, 6 mmol) in ethyl ether (30 ml) was reated in an ice-water bath with excess etheral diazomethane until a yellow color persisted. Nitrogen was bubbled through the mixture for 10 minutes and the solution was concentrated. The residual oil was taken up in ethyl ether and the solution was washed with cold dilute potassium bisulfate and then brine (twice), dried over sodium sulfate and concentrated to give 2.046 g of the desired product as an oil.

e)



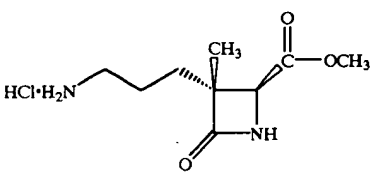
A mixture of the product from step (d) (2.95 g, 8.83 mmol), sodium azide (2.44 g, 35.3 mmol) and tetrabutylammonium iodide (2.45 g, 6.64 mmol) in dimethylformamide (12 ml) was stirred at 60° C. under argon for 16 hours. This material was combined with a second reaction mixture obtained from the product from step (d) (334 mg, 1 mmol). The dimethylformamide was removed in vacuo and the residue was taken up in ethyl acetate and dilute aqeuous lithium chloride. The ethyl acetate layer was washed again with dilute lithium chloride and then brine (twice), dried over sodium sulfate, and concentrated to give 3.53 g of the desired product as a crude viscous oil.

f)



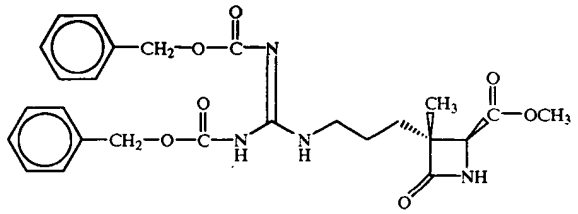
A solution of the product from step (e) (3.53 g) in tetrahydrofuran (30 ml), acetic acid (1.2 ml), and 1.0M tetrabutylammonium fluoride (20 ml) in tetrahydrofuran was stirred at room temperature for 2 hours and then concentrated to a residue which was taken up in ethyl acetate and brine. After extracting, the ethyl acetate layer was washed with brine (twice), dried and sodium sulfate, and concentrated to an oil (9.33 g). Chromatography of this oil over 200 g of silica gel using ethyl acetate:hexanes (7:3) gave 2.1 g of the desired product as an oil.

g)



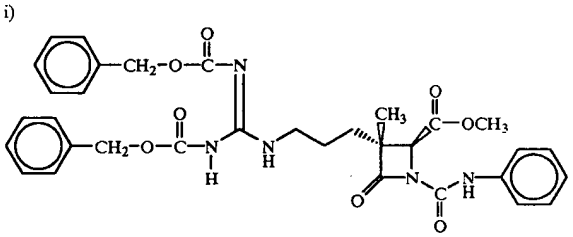
The product from step (f) (678 mg, 3 mmol) was hydrogenated in 20 ml of dioxane and 3.0 ml of aqueous 1M HCl in the presence of 10% palladium on carbon catalyst (237 mg) for 2 hours. After filtration using aqueous dioxane and concentration of the filtrate, the residue was concentrated from dioxane repeatedly to give 974 mg of the desired product as a crude thick gum.

h)



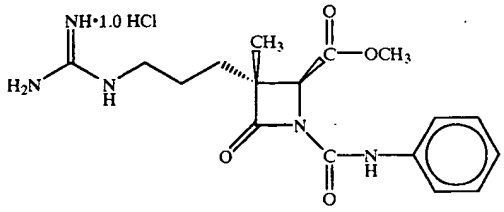
To a solution of the product from step (g) (945 mg) in dry methanol (7 ml) and dry dimethyl ether (7 ml) under argon was added sequentially N,N'-dicarbobenzyloxy—S-methylisothiourea (1.61, 4.5 mmol), triethylamine (1.5 ml, 10.7 mmol) and mercuric chloride (1.22 g, 4.5 mmol). The reaction was stirred at room temperature for 2 hours and the filtered through Celite® using ethyl acetate. Concentration of the filtrate gave a residue which was taken up in ethyl acetate and dilute potassium bisulfate. After two extractions with ethyl acetate, the combined ethyl acetate extracts were washed with brine (twice), dried over sodium sulfate, and concentrated to an oil (2.25 g). Purification by chromatography over silica gel (150 g) using ethyl acetate:hexanes (6:4) gave 1.03 g of the desired product as an oily residue.

i)



To the product from step (h) (582 mg, 1.14 mmol), previously dried by azetroping from tetrahydrofuran and toluene, in tetrahydrofuran (8 ml) at −78° C. under argon was added 1.0 M sodium bis(trimethylsilyl)amide (1.5 ml, 1.5 mmol). The mixture was stirred for 15 minutes and then phenylisocyanate (150 μl, 1.38 mmol) was added. The reaction was warmed to 0° C. over 30 minutes and poured into 8 ml of 10% potassium bisulfate and water. After extraction with ethyl acetate (3 times), the combined ethyl acetate extract was washed with water (twice), dried over sodium sulfate, and concentrated to a viscous oil (0.78 g). Purification by chromatography (silica gel, 60 g) using ethyl acetate:hexanes (35:65) gave 656 mg of the desired product as an oily residue.

j)

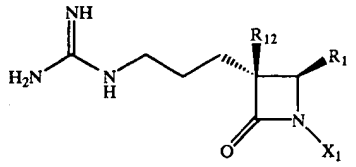


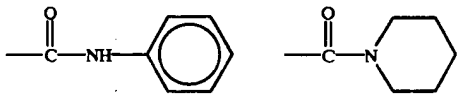
(racemate)

The product from step (i) (636 mg, 1.01 mmol) was hydrogenated in 12 ml of dioxane and 1.0 ml of 1N HCl (1 mmol) in the presence of 10% palladium on carbon catalyst

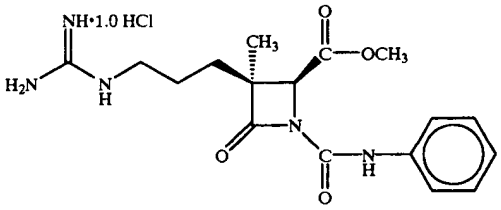
(223 mg) at 1 atmosphere of hydrogen for 2 hours. After filtration using aqueous dioxane, the filtrate was concentrated to a residue which was lyophilized from aqueous acetonitrile to give 336 mg of the desired product as a white hydroscopic solid; IR(KBr) 1775 cm^{-1}; $(M+H)^+$=362.

The following additional compounds of formula II were also prepared



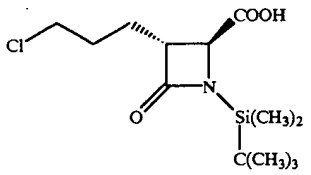
Ex	X_1	R_1	R_{12}	salt	stereochemistry	$(M+H)^+$
167			CH_3	1.0 HCl	racemate	415

EXAMPLE 168

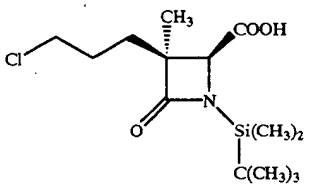


(racemate)

a)

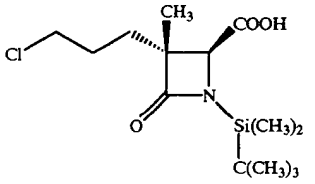


b)



and

3α-methyl epimer

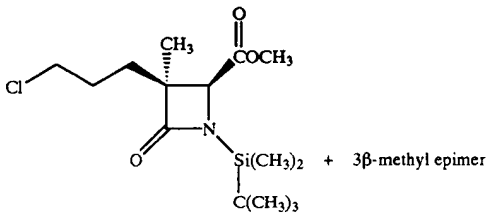


3β-methyl epimer

To diisopropylamine(8.9 ml, 63.5 mmol) in tetrahydrofuran (50 ml) at -20° C. under argon was added 22.6 ml of 2.5 M n-butyl lithium in hexane (56.6 mmol). The mixture was stirred for 15 minutes and cooled to -70° C. A solution of the racemic compound from Example 166 part (a) (6.49 g, 28.3 mmol) in tetrahydrofuran (30 ml) was added over 5 minutes and the reaction was warmed to -20° C. over 15 minutes. A solution of 1-chloro-3-iodopropane (6.3 ml, 58.7 mmol) in tetrahydrofuran (10 ml) was added and the reaction was stirred between -20° C. and 0° C. for 30 minutes. Dry ethyl ether (125 ml) was added and the reaction was poured into a mixture of ice, 1N HCl (130 ml), and ethyl ether. After separation, the aqueous layer was extracted with ethyl ether (twice). The combined ethyl ether extract was washed with brine (twice), dried over sodium sulfate, and concentrated to an oil, which was concentrated from hexanes (5×) to give 7.61 g of the desired product as an oil.

To diisopropylamine (4.7 ml, 33.5 mmol) in tetrahydrofuran (25 ml) at -20° C. under argon was added 12 ml of 2.5 M n-butyl lithium in hexane (30 mmol). The mixture was stirred for 15 minutes and cooled to -70° C. A solution of the product from step (a) (4.50 g, 14.7 mmol), previously dried by azetroping from toluene and tetrahydrofuran, in tetrahydrofuran (20 ml) was added over 5 minutes and the reaction was warmed to -20° C. over 15 minutes. A solution of methyl iodide (1.90 ml, 30.5 mmol) in tetrahydrofuran (6 ml) was added and the reaction was stirred between -20° C. and 0° C. for 30 minutes. Dry ethyl ether (70 ml) was added and the reaction was poured into a mixture of ice, 1N HCl (66 ml), and ethyl ether. After separation, the aqueous layer was extracted with ethyl ether (twice). The extracts were combined, washed with brine (twice), dried over sodium sulfate, and concentrated to an oil, which was concentrated from hexanes (5×) to give 4.60 g of an oil consisting of the 3α-methyl epimer and the corresponding 3β-methyl epimer in a ratio of 4:1.

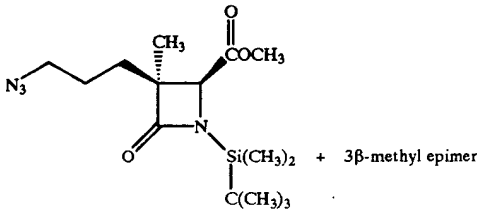
c)



3α-methyl epimer

Treatment of the product mixture from part (b) (1.24 g) with diazomethane according to the procedure of Example 166 step (d) gave 1.36 g of an oil consisting of the 3α-methyl epimer and the corresponding 3β-methyl epimer in a ratio of 4:1.

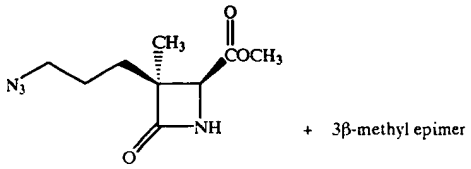
d)



3α-methyl epimer

Treatment of the product mixture from part (c) (1.35 g) with sodium azide and tetrabutylammonium iodide in dimethylformamide according to the procedure of Example 166 step (e) gave 1.21 g of a crude oil containing the 3α-methyl and 3β-methyl epimers.

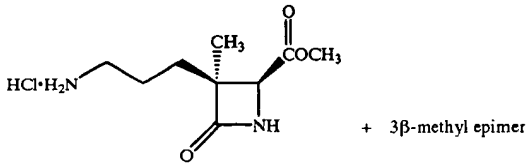
e)



3α-methyl epimer

Treatment of the crude product mixture from part (d) (1.21 g) in tetrahydrofuran (10 ml) with 5.0 ml of 1.0 M tetrabutylammonium fluoride in tetrahydrofuran and acetic acid (0.3 ml) according to the procedure of Example 166 step (f) gave, after chromatography on 100 g of silica gel using ethyl acetate:hexanes (1:1), 343 mg of an oil consisting of the 3α-methyl epimer and the corresponding 3β-methyl epimer in a ratio of (86:14). An additional 256 mg of the mixture was obtained in the chromatography.

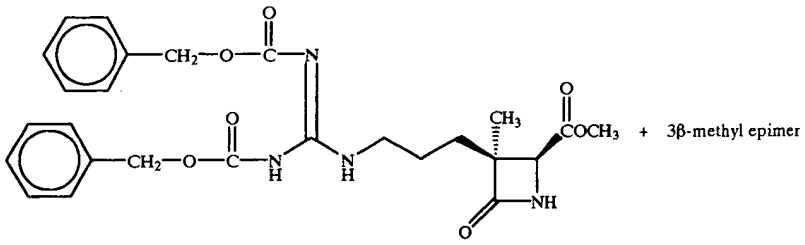
f)



3α-methyl epimer

Hydrogenation of the product mixture from part (e) (335 mg) as described in Example 166 step (g) and concentration of the product from dioxane gave 469 mg of the crude product mixture as a gummy residue.

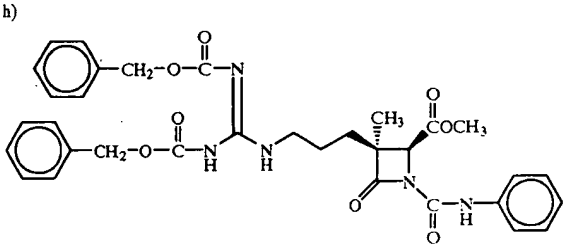
g)



3α-methyl epimer

Treatment of the crude product mixture from part (f) (460 mg) with N,N'-dicarbobenzyloxy—S-methylisothiourea and mercuric chloride as described in Example 166 step (h) gave, after chromatography over 75 g of silica gel using ethyl acetate:hexanes (6:4), 541 mg of a gummy residue consisting of the 3α-methyl epimer and the corresponding 3β-methyl epimer in a ratio of (86:14).

h)

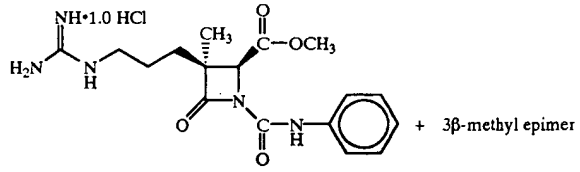


3α-methyl epimer

and the 3β-methyl epimer

Treatment of the product mixture from part (g) (500 mg) with sodium bis(trimethylsilyl) azide and phenylisocyanate as described in Example 166 step (i) gave, after chromatography over 50 g of silica gel using ethyl acetate:hexanes (35:65), 525 mg of gummy residue consisting of 3α-methyl epimer and the corresponding 3β-methyl epimer in a ratio of (86:14).

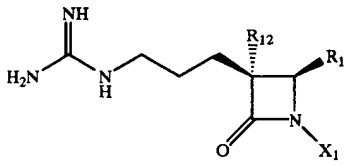
i)

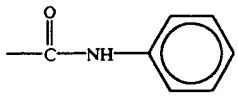
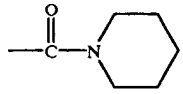
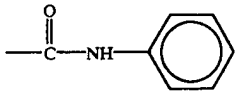


3α-methyl epimer (racemate)

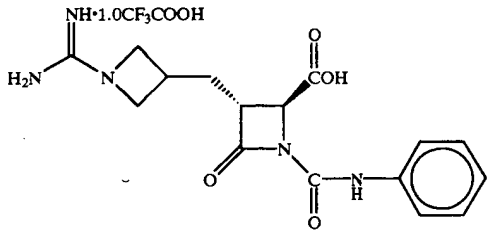
The product mixture from part (h) (457 mg) was hydrogenated in 9 ml of dioxane and 0.73 ml of 1.0N HCl in the presence of 10% palladium on carbon catalyst (160 mg) at 1 atmosphere for 2 hours. After filtration using aqueous dioxane, the filtrate was concentrated to a residue which was lyophilized from aqueous acetonitrile to give 249 mg of a white hydroscopic solid consisting of 3α-methyl epimer and the corresponding 3β-methyl epimer in a ratio of (88:12); IR(KBr) 1775 cm^{-1}; $(M+H)^+$=362.

The following additional compounds of formula III were also prepared



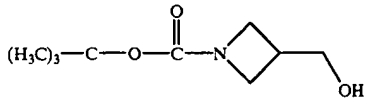
Ex	X_1	R_1	R_{12}	salt	stereochemistry	$(M + H)^+$
169			CH_3	1.0 HCl	racemate	415
170		$—CO_2H$	CH_3	—	racemate	348

EXAMPLE 171

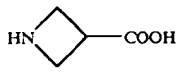


(homochiral)

a)

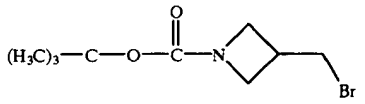


The azetidine carboxylic acid



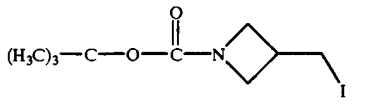
was prepared from epichlorohydrin according to the procedure of A. G. Anderson Jr. and R. Lok, J. Org. Chem., Vol. 37, p. 3953, (1972). This azetidine carboxylic acid was then converted to the desired product according to the procedure of T. L. Hansen et al., WO97/23508.

b)



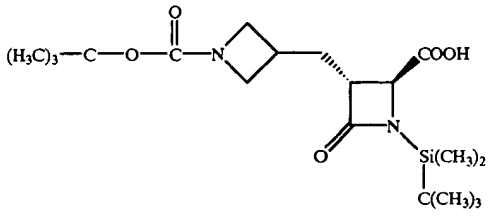
A solution of triphenylphosphine (1.57 g, 6 mmol) in methylene chloride (6 ml) was added dropwise to a stirred solution of the product from part (a) (936 mg, 5 mmol) and carbon tetrabromide (2.32 g, 7 mmol) in methylene chloride 910 ml) at 0° C. under argon. The reaction was then stirred at room temperature for 16 hours. The reaction was concentrated in vacuo and the residue was triturated with ethyl ether. Filtration and evaporation of the filtrate gave 3.34 g of an oily residue which was chromatographed over silica gel by eluting with methylene chloride and then methylene chloride:ethyl acetate (19:1) to give 1.02 g of the desired product as an oily residue.

c)



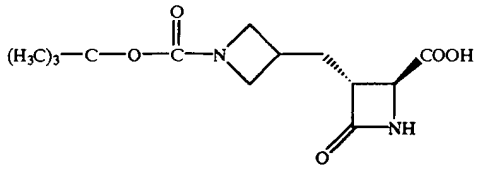
A mixture of the product from part (b) (1.00 g, 4 mmol) and sodium iodide (1.80 g, 12 mmol) in dry acetonitrile (10 ml) under argon was stirred at 65° C. for 2.5 hours, cooled to room temperature and concentrated in vacuo. The residue was taken up in ethyl acetate and water and the ethyl acetate layer was washed with water (twice), dilute sodium thiosulfate, and water (twice), dried over sodium sulfate, and concentrated to give 1.18 g of the desired product as an oily residue.

d)



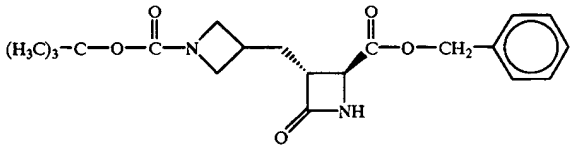
To diisopropylamine (0.63 ml, 4.5 mmol) in tetrahydrofuran (3.5 ml) at −20° C. under argon was added 1.6 ml of 2.5 M n-butyl lithium in hexane (4 mmol). The mixture was stirred for 15 minutes and cooled to −70° C. A solution of (4S)-N-(t-butyldimethylsilyl) azetidine-2-one-4-carboxylic acid (459 mg, 2.0 mmol) [Baldwin et al, Tetrahedron, Vol. 46, p. 4733–4748, 1990] in tetrahydrofuran (2.5 ml) was added over 3 minutes and the reaction was warmed to −20° C. over 15 minutes. A solution of the product from part (c) (1.19 g, 4 mmol) in tetrahydrofuran (3 ml) was added and the reaction was stirred between −20° C. and −30° C. for 1.5 hours and then at −20° C. for 16 hours. The reaction was warmed to 0° C. and quenched by the addition of 5% potassium bisulfate and then ethyl acetate. After extraction with ethyl acetate (three times), the ethyl acetate extracts were combined, washed with brine, dried over sodium sulfate, and concentrated to an oily residue. The residue was dissolved in ethyl ether and washed with saturated sodium bicarbonate (twice). The combined sodium bicarbonate extract was washed with ethyl ether and then layered with ethyl acetate. The pH was adjusted to 2.2 (10% potassium bisulfate) and after extraction with ethyl acetate (three times), the acidic ethyl acetate extract was washed with brine, dried over sodium sulfate, and concentrated to give 624 mg of the desired product as a crude oil.

e)



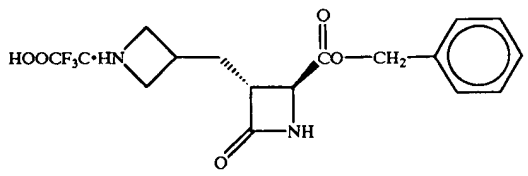
To the crude product from part (d) in tetrahydrofuran (3 ml) at 0–5° C. under nitrogen was added 2.7 ml of 1M tetrabutylammonium fluoride in tetrahydrofuran. The reaction was stirred for 1.5 hours at room temperature and then the solvent was removed in vacuo and the residue was taken up in ethyl acetate, water and 10% potassium bisulfate (11 ml). After extraction with ethyl acetate (three times), the extracts were combined, washed with small amounts of water (twice), and brine, dried over sodium sulfate, and concentrated to give 470 mg of crude desired product as an amorphous residue.

f)



A solution of the product from part (e) (466 mg), benzyl bromide (0.84 ml,.7.1 mmol) and sodium bicarbonate (239 mg, 2.84 mmol) in dry dimethylformamide (4 ml) was stirred at room temperature under nitrogen for 16 hours. The reaction was diluted with ethyl acetate and water, and the aqueous layer was extracted with ethyl acetate (twice). The ethyl acetate extracts were combined, and washed with dilute potassium bisulfate, water (twice) and brine, dried over sodium sulfate, and concentrated to give 563 mg of an oil. This oil was chromatographed over silica gel by eluting with methylene chloride and then methylene chloride:ethyl acetate (1:1) to give 407 mg of the desired product as an amorphous residue.

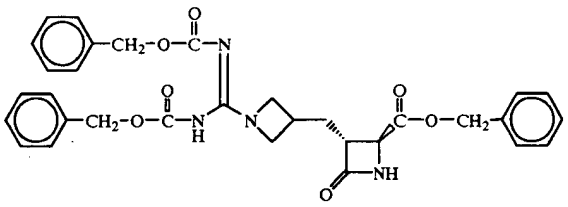
g)



Trifluroacetic acid (1 ml) was added to a stirred solution of the product from step (f) (300 mg, 0.80 mmol) in methylene chloride (3 ml) at 0–5° C. After 5 minutes, the reaction was stirred at room temperature for 1 hour and then

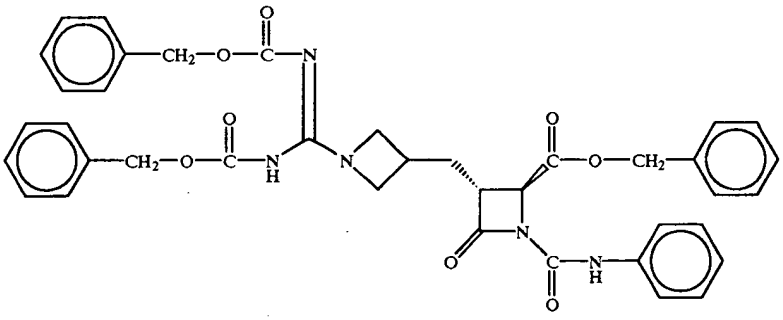
concentrated in vacuo to give the desired product as a residue.

h)



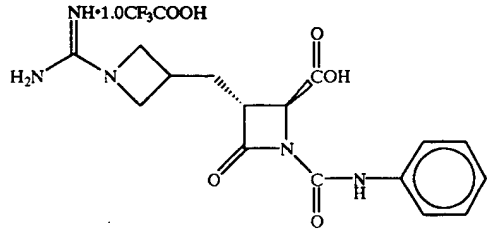
To a solution of the product from part (g) (0.80 mmol) in dry dimethylformamide (3 ml) under argon was added sequentially N,N'-dicarbobenzyloxy-S-methylisothiourea (430 mg, 1.20 mmol), triethylamine (0.45 ml, 3.23 mmol), and mercuric chloride (326 mg, 1.20 mmol). The reaction was stirred at room temperature for 2.5 hours and then filtered through Celite® using ethyl acetate. The filtrate was washed with dilute aqueous potassium bisulfate (twice) and brine, dried over sodium sulfate, and concentrated to an oily residue (767 mg). Purification by chromatography over silica gel eluting with methylene chloride:ethyl acetate (6:4) and methylene chloride: ethyl acetate (3:7) gave 342 mg of the desired product as an oily residue.

i)



To the product from part (h) (136 mg, 0.23 mmol), previously dried by azetroping from tetrahydrofuran and toluene, in tetrahydrofuran (2 ml) at −78° C. under argon was added 0.30 ml of 1.0M sodium bis(trimethylsilyl)amide (0.30 mmol). The mixture was stirred for 10 minutes and then phenylisocyanate (31 μl,0.28 mmol) was added. The reaction was warmed to 0° C. over 40 minutes and poured into 2 ml of 10% potassium bisulfate and water. After extraction with ethyl acetate (three times), the ethyl acetate extracts were combined, washed with water (three times), dried over sodium sulfate, and concentrated to give 193 mg of an oil. Purification by chromatography over silica gel eluting with methylene chloride:ethyl acetate (98:2) and then methylene chloride:ethyl acetate (95:5) gave 121 mg of the desired product as an oily residue.

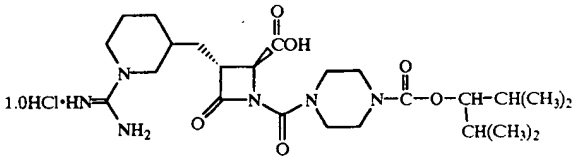
j)



(homochiral)

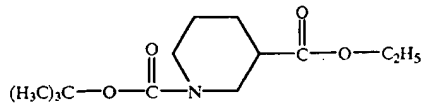
The product from part (i) (115 mg, 0.163 mmol) was hydrogenated in dioxane (4 ml) and 1.0ON HCl (163μl, 0.163 mmol) in the presence of 10% palladium on carbon catalyst (35 mg) at 1 atmosphere for 1 hour. After filtration using aqueous dioxane, the filtrate was concentrated to a residue. This residue was lyophilized from aqueous dioxane to give 89 mg of crude product. Purification by preparative HPLC [YMC S5 ODS 30x250 mm, 25 ml/min, using a gradient (10–40%) of solvent A (10% methanol+90% water+0.1% trifluoroacetic acid) and solvent B (90% methanol+10% water+0.1% trifluoroacetic acid)] gave after lyophilization 31 mg of desired product as a white hydroscopic solid, IR (KBr) 1780 cm^{-1}; (M+H)+=346.

EXAMPLE 172



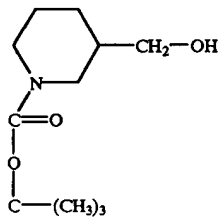
(diastereomeric mixture)

a)



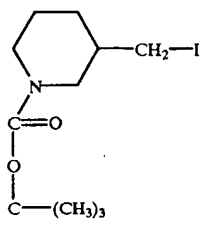
Di-tert-butyl dicarbonate (10.9 g, 50 mmol) was slowly added to a stirred solution of ethyl nipecotate (6.2 ml, 40 mmol) and N,N-diisopropylethylamine (7.0 ml, 40 mmol) in methylene chloride (80 ml) at 0° C. under argon. The cooling bath was removed, dimethylaminopyridine (0.49 g, 4 mmol) was added, and the reaction was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue was taken up in ethyl acetate. The ethyl acetate was washed with dilute HCl(2×) and brine (2×), dried over magnesium sulfate and concentrated to an oil, which was pased through a column of silica gel using hexanes-ethyl acetate (8:2) to provide 10.2 g of the desired product as a colorless oil.

b)



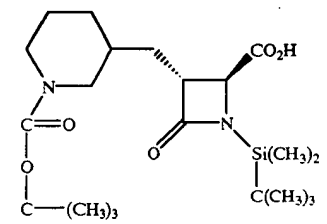
A solution of 1M lithium aluminum hydride in tetrahydrofuran (40.4 ml, 40.4 mmol) was added over 10 minutes to a stirred solution of the product from part (a) (9.92 g, 38.5 mmol) in tetrahydrofuran (120 ml) at 0° C. under argon. After 45 minutes, the ice water cooled reaction was decomposed by the cautious dropwise addition of 5N sodium hydroxide (10 ml). The mixture was stirred for 10 minutes and the semigranular mixture was filtered. The filtrate was concentrated to an oil, which was taken up in ether. The ether was washed with brine, dried over sodium sulfate and concentrated to an oil, which solidified in vacuo to give 7.73 g of the desired product.

c)



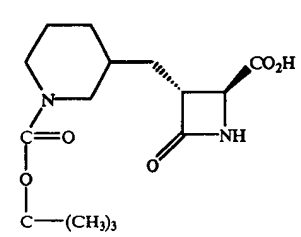
A solution of the product from part (b) (4.1 g, 20 mmol) in 30 ml of methylene chloride was added over 10 minutes to a stirred solution of triphenylphosphine (7.34 g, 28 mmol), imidazole (1.91 g, 28 mmol) and iodine (7.1 g, 28 mmol) in 70 ml of methylene chloride at 0° C. under argon. The cooling bath was removed and the reaction was stirred at room at room temperature for 1 hour and filtered. Concentration of the filtrate gave an oil which was stirred with ethyl acetate for 15 minutes. After filtration, the filtrate was washed with 5% sodium thiosulfate (3×) and then brine, dried over magnesium sulfate, and concentrated to give 11.4 g of crude product, which was chromatographed over silica gel using methylene chloride to afford 6.1 g of the desired product as a colorless oil.

d)



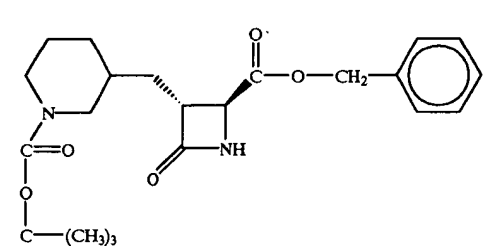
Reaction of (4S)-N-(t-butyldimethylsilyl)azetidine-2-one-4-carboxylic acid (1.15 g, 5 mmol) and the product from part (c) (3.25 g, 10 mmol) according to the procedure of Example 171 step (d) gave 1.98 g of the crude desired product as a foam.

e)



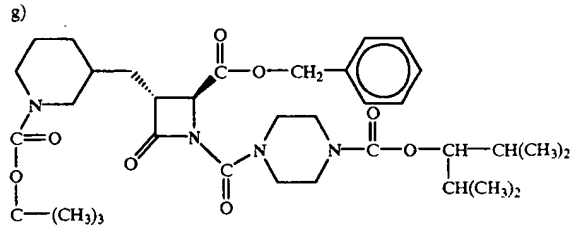
Treatment of the product from part (d) (1.98 g) with tetra-butylammonium fluoride in tetrahydrofuran according to the procedure of Example 171 step (e) gave 1.64 of the crude desired product as an oil.

f)



Treatment of the product from part (e) (1.64 g) with benzyl bromide according to the procedure of Example 171 step (f) gave 1.23 g of the desired product as an oil after silica gel chromatography by eluting with methylene chloride and then methylene chloride/ethyl acetate (6:4).

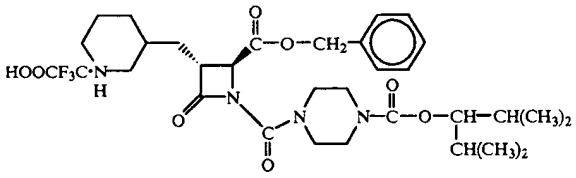
g)



A solution of the product from part (f) (402 mg, 1 mmol), triethylamine (0.28 ml, 2.0 mmol), 1-diisopropylmethoxycarbonyl-piperazine-4-carbonylchloride (436 mg, 1.50 mmol) and dimethylaminopyridine (31 mg, 0.25 mmol) in methylene chloride (4.5 ml) was stirred at room temperature under argon for 8 hours and stored at 0° C. overnight. The reaction was concentrated in vacuo and the residue was taken up in ethyl acetate, 10% potassium bisulfate (4 ml) and water. The ethyl acetate layer was washed again with dilute potassium bisulfate, water (2×), and brine, dried over sodium sulfate and concentrated

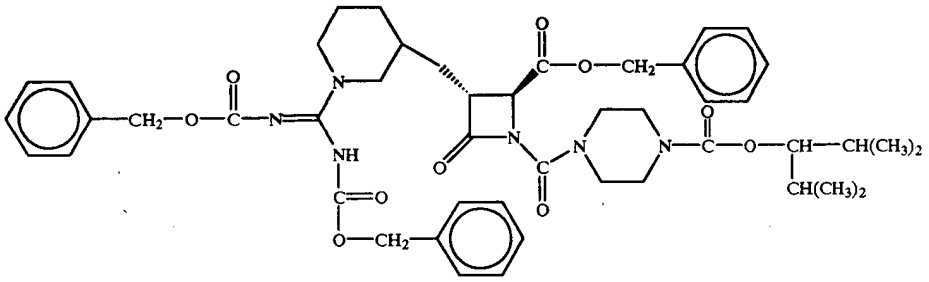
to a viscous oil (779 mg). Chromatography of the oil over silica gel using 10% and then 20% ethyl acetate in methylene chloride provided 618 mg of the desired product as an oil.

h)



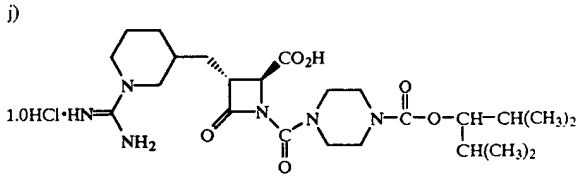
Trifluoroacetic acid (0.75 ml) was added to a stirred solution of the product from part (g) (436 mg, 0.66 mmol) in methylene chloride (3 ml) at 0–5° C. After 5 minutes, the reaction was stirred at room temperature for 2 hours and concentrated in vacuo to a residue, which was further concentrated from methylene chloride (5×) and then chloroform (2×) to give 648 mg of compound of the desired product as an oil.

i)



To a solution of the product from part (h) (0.25 ml) in dimethylformamide (1.5 ml) under argon were added sequentially N,N'-dicarbobenzyloxy-S-methylisothiourea (136 mg, 0.38 mmol), triethylamine (0.21 ml, 1.5 mmol) and mercuric chloride (103 mg, 0.38 mmol). The reaction was stirred at room temperature for 3 hours, diluted with ethyl acetate and filtered through Celite. The filtrate was washed with dilute aqueous potassium bisulfate (2×) and brine (2×), dried over sodium sulfate and concentrated to an oil (280 mg), which was purified by chromatography over silica gel by eluting with 15% and then 20% ethyl acetate in methylene chloride to give 146 mg of the desired product as an oily residue.

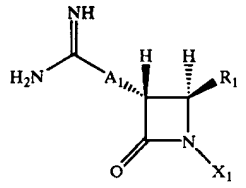
j)

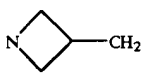
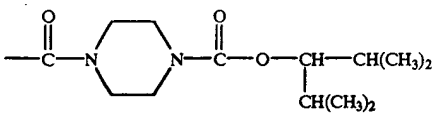
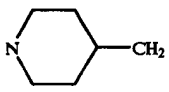
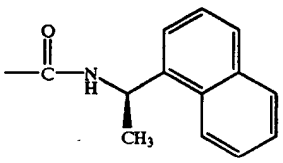
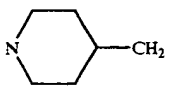
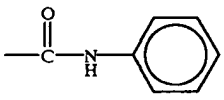
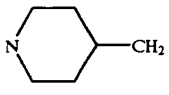
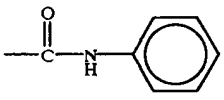
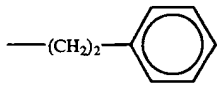
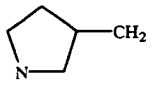
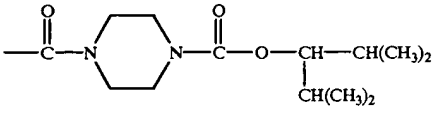


(diastereomeric mixture)

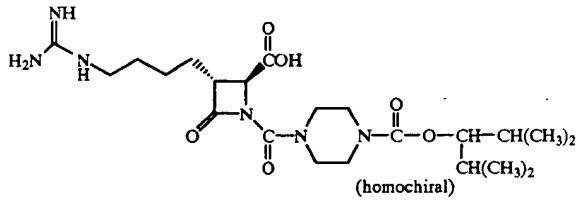
The product from part (i) (141 mg, 0.163 mmol) was hydrogenated in dioxane (5 ml) and 1.0 N HCl (0.163 mmol) in the presence of 10% palladium on carbon catalyst (42 mg) at 1 atmosphere for 1 hour. After filtration using aqueous dioxane, the filtrate was concentrated to remove dioxane, filtered, and lyophilized to give 47 mg of the desired product as a white solid; IR(KBr) 1787 cm^{-}, consisting of a mixture (52:48) of diastereomers as determined by HPLC.

The following additional compounds of formula I were also prepared



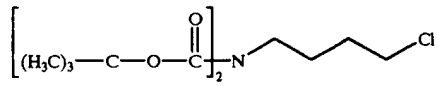
Ex	—A_1—	X_1	R_1	salt	stereo-chem-istry	$(M + H)^+$
173			—CO_2H	—	homo-chiral	481
174			—CO_2H	1.0 HCl	homo-chiral	452
175			—CO_2H	1.0 HCl	homo-chiral	374
176				1.0 HCl	race-mate	434
177			—CO_2H	1.0 HCl	dia-stereo-meric mixture	495

EXAMPLE 178



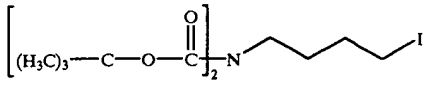
(homochiral)

a)



To a solution of di(tert-butoxycarbonyl)amine (2.17 g, 10 mmol) in dimethylformamide (40 ml) at 0° C. was added 6.8 g of a solution of potassium tert-butylamylate in toluene. The addition was carried out very slowly over 30 minutes. After stirring for one hour at 0° C., 1-chloro-4-iodobutane (2.18 g, 10 mmol) was added dropwise and stirring was continued for 2 more hours. Water (20 ml) and hexane (20 ml) were added for the work-up. The aqueous layer was extracted with additional hexane (3×20 ml). The combined organic layer was washed with 1.0 N ice cold sodium hydroxide, saturated sodium phosphate, monobasic solution, and finally brine. After drying over sodium sulfate and evaporation, 2.76 g of the desired product was obtained as a light yellow oil.

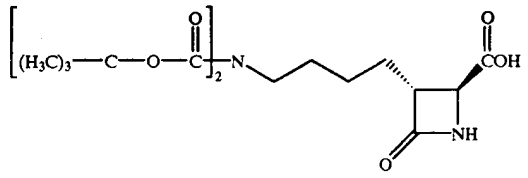
b)



To a solution of the product from part (a) (4.5 g, 15 mmol) in acetone (60 ml) were added sodium iodide (7.3 g, 49 mmol) and sodium bicarbonate (12 mmol). The reaction was refluxed at 60° C. for 12 hours.

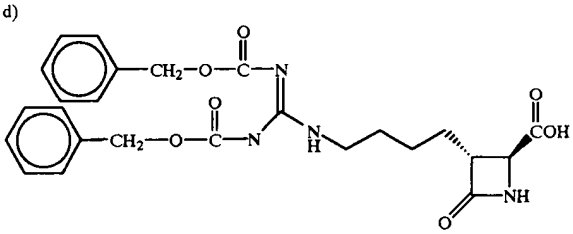
At that time, additional sodium iodide (2 g) and acetone (30 ml) were added and refluxing was continued for another 8 hours. Evaporation and extraction of the residue (oil and solid) with hexane (5×30 ml) and washing of the combined extraction solutions with 2.0 N sodium sulfite and brine, and drying over sodium sulfate yielded after concentration 5.02 g of the desired product as a yellow oil.

c)

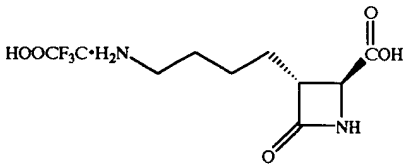


(4S)-N-(tert-Butyldimethylsilyl)azetidine-2-one-4-carboxylic acid (2.30 g, 10 mmol) [Baldwin et al., Tetrahedron, Vol. 46, p. 4733–4748, 1990] dissolved in tetrahydrofuran (10 ml) was added dropwise over 20 minutes to a solution of lithium diisopropylamide (21 mmol) in tetrahydrofuran (60 ml) at −50° C. After the addition was completed, the temperature was allowed to rise to −20° C. at which time a solution of the product from part (b) (4.80 g, 12 mmol) dissolved in tetrahydrofuran (10 ml) was added dropwise. Stirring was continued for 2 hours at −20° C. after the addition was completed. After warminig to 0° C., water (100 ml) was added and the pH was adjusted to 12.5 by the addition of 1.0 N sodium sulfate solution. After stirring for 1 hour at 0° C. the reaction solution was extracted with hexane (50 ml). The aqueous layer was adjusted to pH 3 with 6.0 N HCl and extracted with ethyl acetate (150 ml). The organic phase was washed with brine and dried to give 4.13 g of the desired product as a colorless oil.

d)



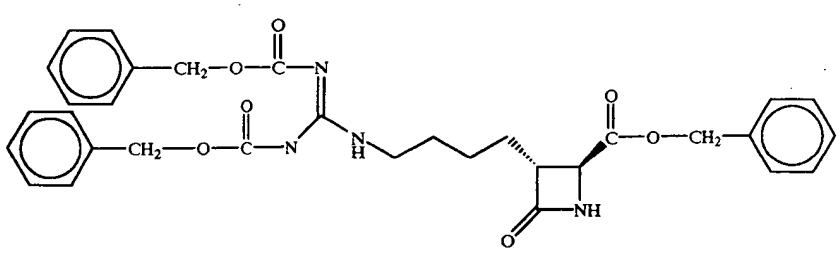
Trifluoroacetic acid (25 ml) was slowly added to the product from part (c) (2.5 g, 5 mmol) dissolved in methyle chloride (50 ml) at 0° C. After stirring for 30 minutes and evaporation, toluene (20 ml) was added to the oily residue. The toluene was evaporated again to remove excess trifluoroacetic acid and give 1.6 g of



which was used for the next step without purification.

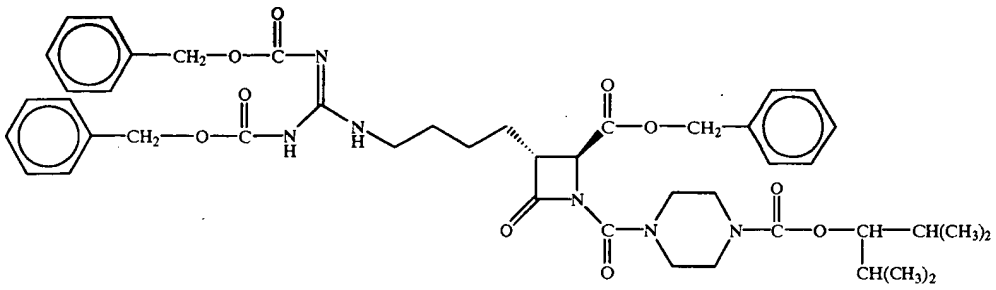
The above trifluoroacetic acid salt (1.6 g) was dissolved in methanol (30 ml) and after cooling to −5° C. the pH was adjusted to 8.5 by adding triethylamine (0.7 ml) followed by N,N'-dicarbobenzyloxy-S-methylisothiourea (2 g, 5.5 mmol). The reaction was stirred for 12 hours at room temperature. The methanol was stripped off in vacuo an the oily residue was taken up in ethyl acetate (20 ml) and water (10 ml). After cooling to 0° C., the pH was adjusted to 3.0 with 2.0 N sodium bisulfate solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2×20 ml). The combined organic layer was washed with brine and extracted with an ice cold saturated sodium bicarbonate solution (3×20 ml). After cooling to 0° C., the aqueous phase was acidified with concentrated HCl to a pH of 3.2 and reextracted with ethyl acetate (3×20 ml). The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated to give 1.95 g of the desired product as a colorless oil.

e)



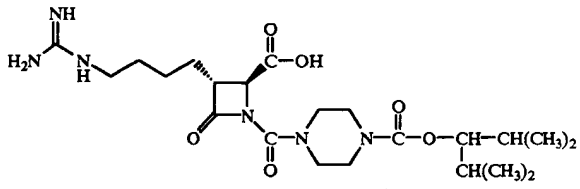
The product from part (d) (497 mg, 1.0 mmol) was dissolved in tetrahydrofuran (5 ml) and butanol (155 μl, 1.5 mmol), dicyclohexylcarbodiimide (210 mg, 1.0 mmol), 4-dimethylaminopyridine (10 mg) and hydroxybenzotriazole (20 mg) were added with stirring. After stirring for 6 hours at room temperature, methylene chloride (5 ml) was added. After filtration, the filtrate was concentrated in vacuo to give 570 mg of crude product as a colorless oil. Purification by flash chromatography on silica gel eluting with ethyl acetate/hexane gave 495 mg of the desired product; IR (KBr) 1745 cm^{-1}.

f)



The product from part (e) (295 mg, 0.5 mmol) was dissolved in methylene chloride (7 ml). After the addition of diisopropylamine (194 mg, 1.5 mmol), 1-diisopropylmethoxycarbonylpiperazine-4-carbonylchloride (218 mg, 0.75 mmol) and dimethylaminopyridine (10 mg), the reaction solution was stirred overnight at room temperature. Additional 1-diisopropylmethoxycarbonylpiperazine-4-carbonylchloride (20 mg) was added and stirring was continued for 4 hours. The reaction was quenched with 10 ml of ice water (pH was adjusted to 4 with potassium sulfate solution) and ethyl acetate. The aqueous layer was extracted with ethyl acetate (2×10 ml) and the combined organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The resulting colorless oily residue was purified by flash chromatography over silica gel eluting with ethyl acetate:hexanes (4:6) to give 392 mg of the desired product; IR(KBr) 1790 cm^{-1}.

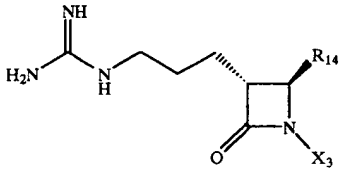
g)

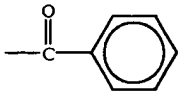
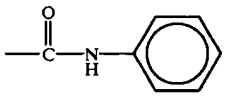
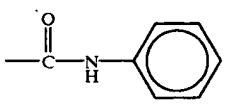


(homochiral)

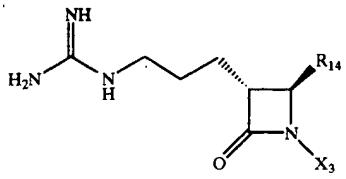
A mixture of the product from part (f) (96 mg, 0.11 mmol, 1N HCl (110 μl, 0.11 mmol), 10% palladium on carbon catalyst (37 mg) in dioxane (2.5 ml) was stirred under a hydrogen atmosphere (hydrogen balloon) at room temperature for 1 hour. The reaction was filtered through a Celite® cake, passed through a polyvinylpyrrolidone resin column, and lyophilized to give 44 mg of the desired product as a white fluffy powder; IR (KBr) 1780 cm^{-1}, 1669 cm^{-1}; $(M+H)^+$=483.3, $(M-H)^-$=481.

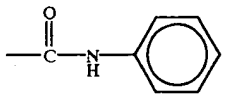
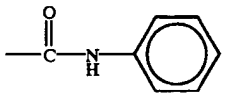
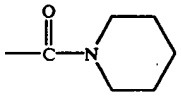
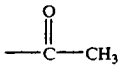
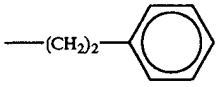
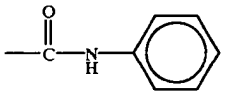
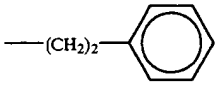
In addition to the compounds prepared by Han in U.S. Pat. No. 5,037,819, the following compounds of formula VI were prepared



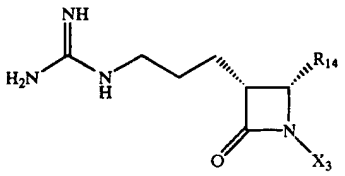
Ex	X_3	R_{14}	salt	stereochemistry	$(M+H)^+$
179		$-CO_2H$	1.0 CF_3CO_2H	homochiral	319
180		$-CO_2H$	1.0 HCl	homochiral	334
181		$-CO_2CH_3$	1.0 HCl	homochiral	348

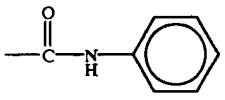
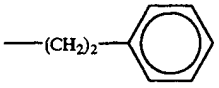
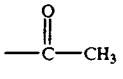
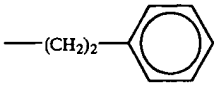
-continued



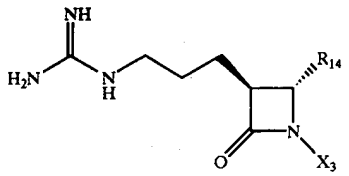
Ex	X_3	R_{14}	salt	stereochemistry	$(M + H)^+$
182		$-CO_2H$	1.0 CF_3CO_2H	racemate	334
183			1.0 HCl	racemate	401
184			1.0 HCl	homochiral	317
185			1.0 HCl	racemate	394

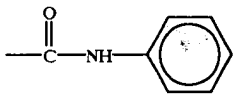
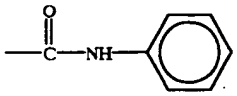
The following additional compounds of formula VI were also prepared



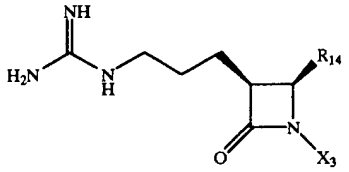
Ex	X_3	R_{14}	salt	stereochemistry	$(M + H)^+$
186			1.0 HCl	racemate	394
187			1.0 HCl	racemate	317

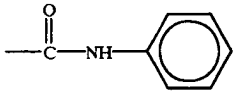
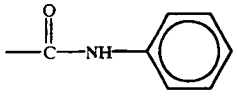
The following additional compounds of formula VI were also prepared



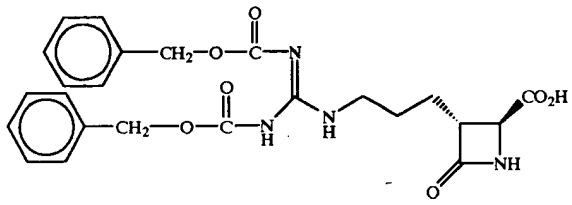
Ex	X_3	R_{14}	salt	stereochemistry	$(M + H)^+$
188		$-CO_2CH_3$	1.0 CF_3CO_2H	homochiral	348
189		$-CO_2H$	—	homochiral	332

The following additional compounds of formula VI were also prepared



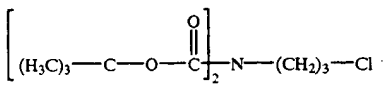
Ex	X_3	R_{14}	salt	stereochemistry	$(M + H)^+$
190		$-CO_2CH_3$	1.0 HCl	racemate	348
191		$-CO_2H$	1.0 CF_3CO_2H	racemate	334

EXAMPLE 192



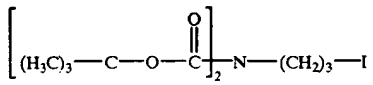
The intermediate of Example 1(b) was also prepared as follows:

a)



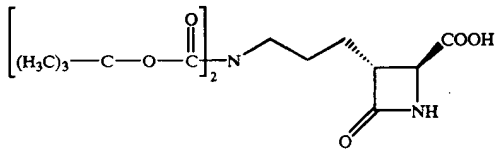
Potassium t-amylate (25% wt in toluene, 164.0 g, 322.6 mmol) was added to a solution of di-t-butyl iminodicarboxylate (70.1 g, 322.6 mmol) in dimethylformamide (500 ml) over a 15 minute period under nitrogen. The resulting white creamy solution was stirred at 0° C. for 40 minutes. 1-Chloro-3-iodopropane (60.0 g, 31. ml, 293.3 mmol) was added and the mixture was stirred at 0° for 3 hours. Hexane (500 ml) and water (300 ml) were added to the mixture. The aqueous layer was separated and extracted with hexane (300 ml). The combined hexane extracts were washed with 1N sodium hydroxide (3x300 ml), saturated sodium hydrogen phosphate (300 ml), half-saturated brine (300 ml), and brine (500 ml) and dried over sodium sulfate. Removal of the sodium sulfate by filtration followed by concentration gave 84.2 g of the desired product as a light yellow oil which was dried under vacuum overnight.

b)



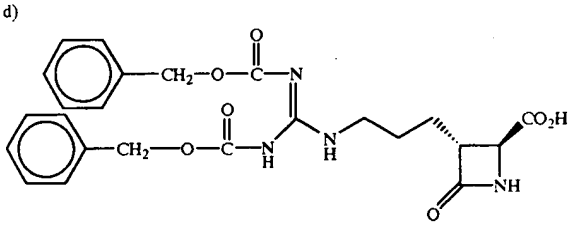
The product from part (a) (55.0 g, 187.2 mmol) was dissolved in acetone (550 ml). Sodium iodide (84.2 g, 561.6 mmol) and sodium bicarbonate (7.9 g, 93.6 mmol) were added. The mixture was stirred at 58° C. (oil bath) under nitrogen for 6 hours and additional sodium iodide (14 g, 93.4 mmol) was added). The reaction mixture was stirred for 12 hours and the acetone was evaporated. Hexane (400 ml) and water (300 ml) were added to the resulting solid. The hexane layer was separated, washed with 5% sodium thiosulfate (300 ml), half-saturated brine (300 ml) and brine (300 ml), dried over sodium sulfate, and filtered through 20 g of a silica gel pad. The silica gel pad was washed with hexane (400 ml). Concentration of the filtrate gave 68.2 g of the desired product as a light yellow oil which was dried under vacuum overnight.

c)



n-Butyl lithium (2.5 M in hexane, 46 ml, 115.0 mmol) was added to a solution of diisopropylamine (11.7 g, 16.2 ml, 115.3 mmol) in tetrahydrofuran (150 ml) at 0° C. under nitrogen. The resulting solution was stirred at 0° C. for 30 minutes then cooled to −30° C. A solution of (4S)-N-(t-butyldimethylsilyl)-azetidine-2-one-4-carboxylic acid (12.0 g, 52.4 mmol) [Baldwin et al, Tetrahedron, Vol. 46, p. 4733–4748, 1990] in tetrahydrofuran (60 ml) was added and the mixture was stirred at −20° C. for 30 minutes. A solution of the iodo product from part (b) (24.0 g, 62.3 mmol) in tetrahydrofuran (30 ml) was added dropwise over a 20 minute period and the resulting mixture was stirred at −20° C. for 2 hours. The reaction mixture was allowed to warm to 0° C. and water (300 ml) was added. The mixture, adjusted to pH 12.5 with 10% sodium bisulfate, was stirred at 0° C. for 30 minutes and then washed with hexane (2×100 ml). The aqueous layer was cooled in an ice-bath and acidified to pH 3.0 by the dropwise addition of 6N HCl. This solution was saturated with sodium chloride and extracted with ethyl acetate (3×150 ml). The combined ethyl acetate extracts were washed with brine (200 ml), dried over sodium sulfate, filtered and concentrated to give a yellow oil. This oil was dissolved in acetonitrile (20 ml) and evaporation of the acetonitrile gave 16.4 g of the desired product as a yellow foam.

d)



The product from part (c) (4.25 g, 11.4 mmol) was added to a 1:2 mixture of trifluroacetic acid/methylene chloride (42 ml). The resulting mixture was stirred at room temperature for 30 minutes under a nitrogen atmosphere. Toluene was added (80 ml) and the mixture was concentrated to a small volume (approximately 15 ml). Additional toluene (80 ml) was added and the mixture was concentrated to dryness to afford a yellow oil.

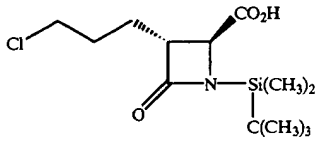
A mixture of triethylamine (3.97 ml, 28.5 mmol) and methanol (42 ml) was added into the above oil at 0° C. Additional triethylamine (0.85 ml, 5.7 mmol) and N,N'-bis (benzyloxycarbonyl)-1-guanylpyrazole (4.31 g, 11.4 mmol) [Wu et al, Synthetic Communications, 23(21), p. 3055–3060 (1993)] were added. The mixture was stirred at room temperature for 11 hours and then concentrated in vacuo at 25° C. to afford a yellow oil.

Ethyl acetate (30 ml) and water (10 ml) were added to this oil followed by acidification to pH 3.2 at 0° C. by the addition of 2M potassium bisulfate which was saturated with sodium chloride. The acidic mixture was poured into a separatory funnel. The layers were separated, and the aqueous layer was washed with ethyl acetate (2×25 ml) while ensuring that the pH of the aqueous solution was in the range of 2.9 to 3.2. The combined ethyl acetate solutions were washed with saturated sodium chloride solution (25 ml)and the product was extracted with saturated sodium bicarbonate (3×25 ml). The combined sodium bicarbonate solutions were washed with ethyl acetate (2×25 ml), acidified to pH 3.2 with concentrated HCl at 0° C., treated with saturated sodium chloride (solid), and finally extracted with ethyl acetate (3×25 ml). The combined ethyl acetate solution was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to afford 4.61 (9.55 mmol) of the desired product as a pale yellow foam.

EXAMPLE 193

The product of Examples 21 and 32 was also prepared as follows:

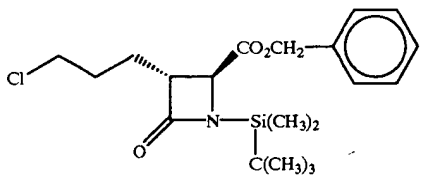
a)



Diisopropylamine (14.05 ml, 0.10 mmol) was added to a dry, three-necked flask equipped with mechanical stirrer and maintained under an argon atmosphere. Tetrahydrofuran (anhydrous, 33 ml) was charged to the flask and while the mixture was stirred and cooled to −20° C., a solution of n-butyl lithium (38.3 ml of a 2.5 M solution in hexanes, 0.096 mol) was added dropwise and the solution was stirred at −20° C. for 10 minutes. A solution of (4S)-N-(t-butyldimethylsilyl)-azetidine-2-one-4-carboxylic acid (10.0 g, 43.6 mmol) [Baldwin et al, Tetrahedron, Vol. 46, p. 4733–4748, 1990] was added slowly while maintaining the temperature at −20° C. and allowed to stir for 30 minutes. A solution of 1-chloro-3-iodopropane (5.6 ml, 52 mmol, 1.2 eq.) in tetrahydrofuran (30 ml) was added over approximately 10 minutes. After stirring at −20° C. for approximately 2 hours, 2.6M potassium bisulfate (75 ml), water (100 ml) and ethyl acetate (100 ml) were added and the mixture was transferred to a separatory funnel. The aqueous layer (pH 2–3) was drawn off and back extracted with ethyl acetate (2×50 ml). The organic solutions were combined and washed sequentially with water (2×50 ml), 10% sodium thiosulfate (1×50 ml) and then with saturated sodium chlo-

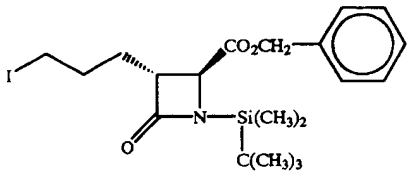
ride to neutral pH. The organic solution was dried over sodium sulfate (15 g), filtered and concentrated to an oil. The oil was seeded with a few crystals of the desired product and placed under high vacuum overnight to dry. The resultant crystalline solid was slurried with hexane (100 ml), filtered, washed with hexane (100 ml) and dried under high vacuum to give 11.5 g of the desired product.

b)



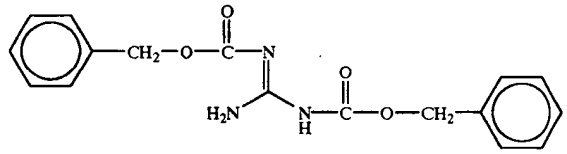
The product from part (a) (7.02 g, 22.9 mmol) was dissolved in methylene chloride (anhydrous, 40 ml) under an argon atmosphere. The solution was stirred and cooled to 0° C., and triethylamine (3.5 ml, 25.2 mmol) was added slowly while maintaining approximately 0° C. Benzylchloroformate (3.6 ml, 25.2 mmol) was added along with an additional 10 ml of methylene chloride to aid stirring. 4-Dimethyl-aminopyridine (2.8 g, 22.9 mmol) was added as a solid in one portion with considerable gas evolution. The solution was allowed to stir at 0° C. for 30 minutes. Additional benzylchloroformate (0.3 ml, 2.5 mmol) was added and the reaction was stirred for an additional 20 minutes. The reaction was quenched with 1M potassium bisulfate (30 ml). The mixture was transferred to a separatory funnel and the layers were separated. The organic layer was washed with 2N potassium bisulfate (20 ml), and the aqueous washes were combined and back extracted with methylene chloride (25 ml). The organic solutions were combined, washed sequentially with water (25 ml), saturated sodium bicarbonate (25 ml), and saturated sodium chloride (2×25 ml), and dried over sodium sulfate (15 g). The methylene chloride solution was filtered through silica (15 g) and the silica was washed with 200 ml 3:1 (volume:volume) hexane/ethyl acetate. The filtrate was concentrated to an oil, evaporated under reduced pressure from toluene (2×25 ml) and dried under vacuum overnight to give 8:12 of the desired product.

c)

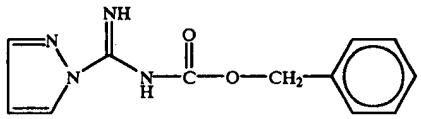


A solution of the product from part (b) (10.55 g, 26.6 mmol) in 4-methyl-2-pentanone (40 ml) was stirred under an argon atmosphere. Sodium iodide (20 g, 133 mmol) was added and the mixture was heated at approximately 110° C., protected from light, for 7 hours. The mixture was cooled to ambient temperature, diluted with hexane (100 ml) and filtered through a plug of Celite®. The Celite® was washed with hexane (2×50 ml). The filtrates were combined, washed with sodium thiosulfate (50 ml) and then with water (50 ml). The aqueous washes were back extracted with ethyl acetate (100 ml). The organic solutions were combined and concentrated to an oil, dissolved in hexane (50 ml) and filtered through a plug of silica. The silica was washed with hexane (200 ml) and then with 4:1 (volume:volume) hexane/ethyl acetate (500 ml). The hexane/ethyl acetate solution was concentrated to give 11.84 g of the desired product as an oil.

d)

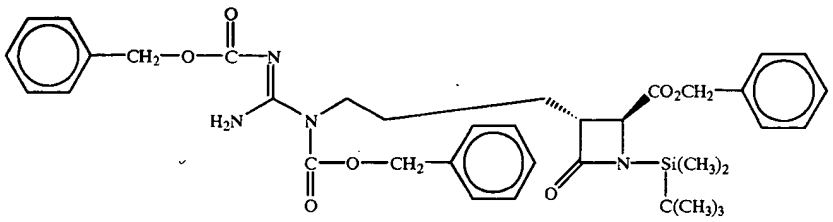


The compound of the formula:



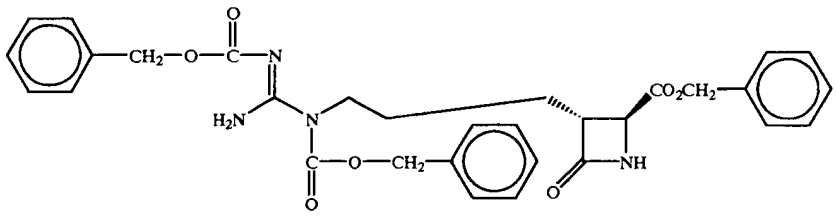
(5 g, 20.5 mmol) and tetrahydrofuran (27 ml, anhydrous) were charged to a dry flask under nitrogen. The solution was stirred and cooled to 0° C. Sodium hydride (2.62 g, 65.5 mmol, 3.2 equivalents of a 60% dispersion in mineral oil) was charged to the flask slowly (exotherm). The suspension was stirred at 0° C. N-(Benzyloxycarbonyloxy)succinimde (8.2 g, 32.8 mmol, 1.6 eq.) was added portionwise maintaining a temperature of approximately 0° C. The cooling was removed and the reaction was allowed to warm to room temperature. After 1 hour, an additional amount of N-(benzyloxycarbonyloxy)succinimide (1 g, 0.2 eq) was added and the reaction was stirred at room temperature overnight. The reaction was worked up by cooling to approximately 0° C. and quenched slowly by the addition of 13% aqueous ammonium chloride. The layers were separated, and the aqueous layer was back extracted with ethyl acetate (3×20 ml). The combined organic solution was washed sequentially with 13% aqueous ammonium chloride (5 ml), water (15 ml) and saturated sodium chloride (2×15 ml). The organic solution was dried over sodium sulfate, filtered and concentrated. The resulting crude oil was charged to a flask with a 2M solution of ammonia in methanol (51 ml, 101 mmol, 5 eq) and allowed to stir at ambient temperature overnight. The reaction was worked up by concentration under reduced pressure, followed by coevaporation with hexanes (2×25 ml). The resultant material was dissolved in methylene chloride (50 ml) and washed with water (50 ml). The aqueous layer was back extracted with methylene chloride (2×50 ml). The organic extracts were combined and washed with water (25 ml) and saturated sodium chloride (25 ml), dried over sodium sulfate, filtered and concentrated to a solid. The solid was crystallized from ethyl acetate to give 4.65 g of the desired product as white crystals.

e)



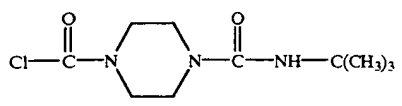
The product from part (d) (6.01 g, 18.37 mmol) was dissolved in 1-methyl-2-pyrrolidinone (anhydrous, 10 ml) and warmed to 35–40° C. under an argon atmosphere. Potassium carbonate (finely ground and dried, 12.7 g, 92 mmol) was added and the mixture was allowed to stir for 25 minutes. A solution of the product from part (c) (9.5 g, 18.37 mmol) in 1-methyl-2-pyrrolidone (10 ml) was added and the reaction mixture was allowed to stir for 8 hours at 35–40° C. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (100 ml) and filtered into a separatory funnel. The mixture was washed with 1M potassium bisulfate (84 ml) and the layers were separated. The aqueous layer was back extracted with ethyl acetate (50 ml). The organic solutions were combined, washed sequentially with water (50 ml), 10% sodium thiosulfate (50 ml) and saturated sodium chloride (50 ml), dried over sodium sulfate, filtered and concentrated to an oil. The oil was dissolved in 50 ml. 1:1 (volume:volume) hexane/ethyl acetate and filtered through silica. The filtrate was concentrated to give 12.88 g of the desired product as a crude oil that was used without further purification.

f)

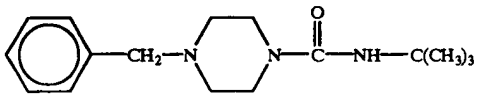


The crude oil product from step (e) was dissolved in acetonitrile (50 ml) and water (5 ml). Ammonium fluoride (3.4 g, 92 mmol) and glacial acetic acid (5.25 ml, 92 mmol) were added and the mixture was stirred for 30 minutes. The reaction was diluted with ethyl acetate (150 ml), transferred to a separatory funnel and washed with saturated sodium bicarbonate (30 ml). The layers were separated and the aqueous layer was back extracted with ethyl acetate. The organic solutions were combined, washed with saturated sodium chloride, dried over sodium sulfate, filtered and concentrated to an oil. The oil was dissolved in ethyl acetate (30 ml) and warmed to 60° C. Hexane (25 ml) was added and the mixture was allowed to cool slowly with stirring and seeding. As crystallization occurred, hexane (75 ml) was added portionwise. The resultant solid was filtered, washed with hexane, and dried to give 8.91 g of the desired product.

g)

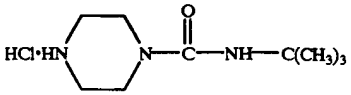


A 2 liter dried flask equipped with a mechanical stirrer and argon inlet was charged with 1-benzylpiperazine (40 ml, 230 mmol) and toluene (250 ml). With vigorous stirring t-butyl isocyanate (27 ml, 236 mmol) was added in rapid dropwise fashion over 15 minutes. The product precipitated to form a thick slurry. The slurry was stirred over an hour to reach 25° C. Heptane (570 ml) was added to the slurry over 30 minutes. The flask was stoppered and placed in a cold room (5° C.) for 4 hours. The product was collected by filtration, rinsed with heptane (1×200 ml) and air dried to give 61.0 gof the piperazine of the formula:



as a white solid.

A 500 ml flask equipped with a magnetic stir bar and a sparging tube was charged with methanol (200 ml). The flask was cooled to 1° C. and with stirring acetyl chloride (5.7 ml, 79.9 mmol) was added over 10 minutes. The solution was allowed to reach room temperature and the above piperazine (20.0 g, 72.6 mmol) was added. Palladium hydroxide on carbon (8.0 g, moisture content less than or equal to 50%) was added and the mixture was then sparged with argon for 10 minutes. The reaction mixture was then sparged with hydrogen. After 3.5 hours, HPLC indicated the starting material was consumed completely. The mixture was filtered through Celite® and the Celite® rinsed with methanol (60 ml). The filtrate was concentrated until solid started to crystallize (175 ml methanol collected). Isopropyl alcohol (200 ml) was added slowly with manual stirring. The mixture was concentrated to a solid/liquid mixture (153 g weight). The mixture was allowed to stand for 2 hours. The product was collected by filtration, washed with isopropyl alcohol (1×30 ml) and air dried to yield 14.0 g of the hydrochloride salt of the formula:

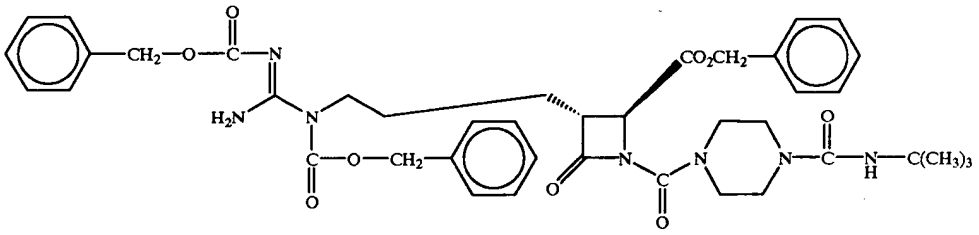


as a light yellow solid.

A 100 ml, three-necked flask with a magnetic stir bar and a sparging tube was charged with this hydrochloride salt (5.0 g, 22.6 mmol) and anhydrous methylene chloride (50 ml). 1,8-Diazabicyclo[5.4.0]undec-7-ene (6.7 ml, 45.1 mmol) and pyridine (1.8 ml, 22.6 mmol) were added to the mixture. The mixture became homogeneous. The mixture was sparged with dry carbon dioxide gas at room temperature for 1 hour.

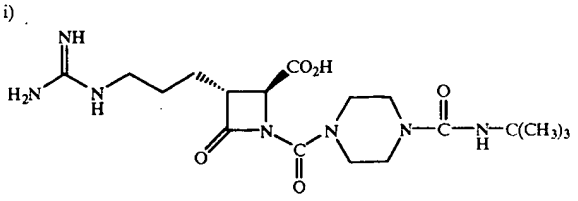
A dry 500 ml flask with a magnetic stir bar was charged with thionyl chloride (4.9 ml, 67.7 mmol) and anhydrous methylene chloride (25 ml). The solution was cooled to −10° C. and dimethylformamide (0.17 ml, 2.26 mmol) was added. The above carbon dioxide sparged mixture was added via cannula under carbon dioxide pressure over 35 minutes. The flask was rinsed with methylene chloride (5 ml) and the rinse was added to the reaction. The reaction was stirred at −10° C. for 30 minutes. The reaction mixture was poured into 0.5 M HCl (75 ml) and shaken vigorously. The organic layer was collected, dried over magnesium sulfate, filtered and concentrated in vacuo to give 4.95 g of the desired product as a light yellow solid.

h)



The product from step (f) (11.84 g, 20.7 mmol) was dissolved in anhydrous methylene chloride (100 ml) under argon with stirring. The carbamoyl chloride product from step (g) (7.47 g, 26.9 mmol, 1.3 eq.), triethylamine (4.6 ml, 33.1 mmol, 1.6 eq.), and 4-dimethylaminopyridine (0.76 g, 6.2 mmol, 0.3 eq) were added, and the reaction was allowed to stir at ambient temperature overnight. The reaction was poured into 0.5 N HCl (110 ml), the layers were separated, and the organic layer was washed with a second portion of 0.5 N HCl. The acidic aqueous layers were back extracted with methylene chloride (50 ml) and combined with the main organic portion. The combined organic layers were washed with saturated sodium bicarbonate (100 ml) and saturated sodium chloride, and dried over sodium sulfate. The solution was filtered and concentrated to give 17.0 g of the desired product as a crude white solid.

i)



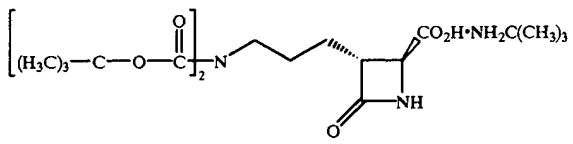
The crude product from part (h) (17.0 g) was dissolved in absolute ethanol (350 ml) with stirring. The solution was

sparged with argon and 10% palladium on carbon catalyst (1.7 g, 50% by weight water) was added in one portion followed by additional argon sparging. The solution was sparged with hydrogen for 2 minutes, and then placed under atmospheric hydrogen pressure (balloon). Two additional charges of palladium on carbon catalyst (1.7 g each) were added to the reaction, along with a repeat of the sparging procedure. The reaction was judged complete in approximately 4 hours (HPLC analysis). The reaction was sparged with argon for 5 minutes and filtered through a packed pad of Celite®. The Celite® was washed with ethanol (2×125 ml). The combined ethanol filtrates were concentrated to approximately 50 g and allowed to stir for 4 days. Crystals formed in the flask. The crystals were filtered, washed with absolute ethanol (25 ml) and dried to give 7.74 g of the desired product as white crystalline material. This material was further purified by warming in 95% ethanol to approximately 40° C. for 30 minutes, followed by cooling, filtration and drying.

EXAMPLE 194

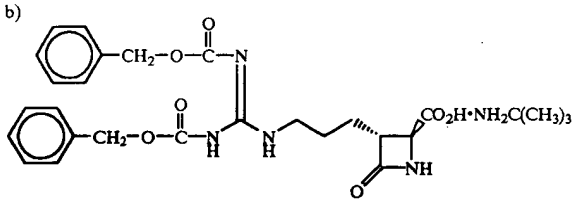
The product of Examples 21, 32 and 193 was also prepared as a zwitterion or inner salt as follows:

a)



A dry, 3-necked, 12-L flask (flask A) was charged with 769.4 g of (4S)-N-(t-butyldimethylsilyl)azetidinone carboxylic acid followed by 6 L of dry tetrahydrofuran. A separate 3-necked, 5-L flask (flask B) was charged with 1537.8 g of the iodo product from Example 192 step (b) followed by 2 L of dry tetrahydrofuran. Under a nitrogen atmosphere, 4 L of dry tetrahydrofuran was charged into a 22-L flask (flask C) followed by 3.69 L of lithium diisopropylamide. The solution of lithium diisopropylamide was cooled to −30 to −35° C. While maintaining the temperature at less than −20° C., the contents of flask A were added to flask C. The mixture was stirred at −20 to −25° C. for 30 to 60 minutes and cooled to −35° C. to −40° C. The contents of flask B were then added portionwise over 25 to 45 minutes while maintaining the internal temperature of flask C at less than about −20° C. The resulting mixture was stirred for 2 to 3 hours between −20° C. and −23° C. The reaction was quenched by the addition of 6 L of cold water while maintaining the internal batch temperature at −20° C. to +5° C. After stirring for an additional 15 to 30 minutes to ensure removal of the silyl protecting group, the pH was adjusted to 8.0 by the addition of cold 6N HCl(1.69 L). The reaction mixture was transferred to a phase splitter and the top organic layer was discarded. The aqueous layer was washed twice with 4L portions of hexane. The aqueous phase was cooled to about 0° C. and treated with 6N HCl (about 400 ml) until the pH was 3.0. The batch temperature was maintained at less than 50 during this operation. The cloudy aqueous phase was extracted three times with 4 L portions of ethyl acetate. The combined organic extracts were washed with brine (3×3L) and concentrated to an oil. The oil was redissolved in 8 L of fresh ethyl acetate, transferred to a 22-L flask, and cooled under nitrogen. While maintaining the batch temperature at less than 8° C., tert-butylamine was added and the resulting mixture was stirred overnight at room temperature. The mixture was concentrated to a yellow slurry, treated with 6L of methyl tert-butyl ether and stirred for 3 hours at room temperature. The mixture was filtered and the filter cake was washed with methyl tert-butyl ether (1.5 L) and dried to a constant weight of 805.9 g of the desired product.

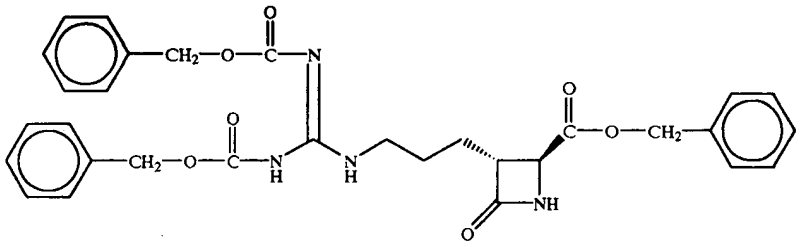
b)



Trifluroacetic acid (31.1 ml, 403.9 mmol, 18 equivalents) was added dropwise to a suspension of the product from part (a) (10.0 g, 22.44 mmol) in methylene chloride (40 ml) at between −5° C. to +5° C. under nitrogen. The resulting light yellow clear solution was stirred at 0° C. until less than 1% of the mono tert-butoxycarbonyl intermediate was detected by HPLC (about 4 to 7 hours). The methylene chloride and trifluoroacetic acid were removed in vacuo at room temperature. Toluene (30 ml) was added to the residue and then removed in vacuo. The residue was treated with isopropyl alcohol (10 ml) followed by toluene (20 ml) and the resulting solution was concentrated in vacuo to an oil. This step was repeated one time.

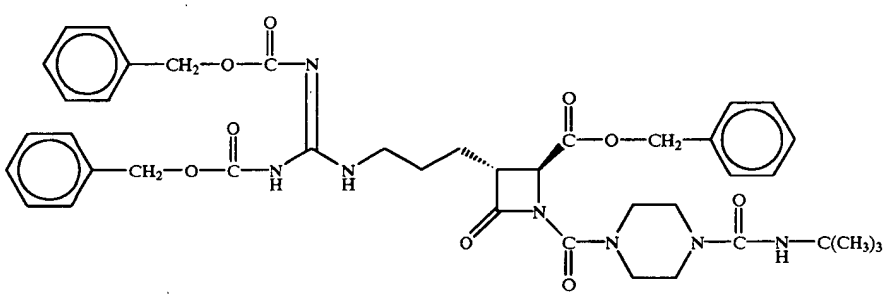
Isopropyl alcohol (50 ml) was added to the above oil (about 23 g) and the resulting solution was cooled to 0° C. The pH was adjusted to 8.5 to 9.0 by the dropwise addition of triethylamine between −5° C. to 5° C. (18 ml of triethylamine was used in this procedure). N,N'-Bis (benzyloxycarbonyl)-1-guanylpyrazole (8.07 g, 21.32 mmol, 0.95 equivalents) was added in one portion and the cooling bath was removed. The mixture was stirred under nitrogen at room temperature for approximately 30 hours until the ratio of product/pyrazole was greater than 25:1 as determined by HPLC. The solvent was removed in vacuo at room temperature to afford approximately 42 g of yellow oil. This oil was diluted with ethyl acetate (70 ml) and water (70 ml), and cooled to 0° C. The pH of the solution was adjusted to 3.0 with 2M potassium bisulfate and treated with sodium chloride until saturated. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2×60 ml). The combined ethyl acetate layers were washed with saturated sodium chloride solution (2×60 ml), dried over sodium sulfate and filtered. The solvent was evaporated to give a yellow oil (14.4 g) which was redissolved in ethyl acetate (40 ml). The resulting clear yellow solution was warmed to 36–40° C. and treated dropwise with tert-butylamine (3.3 ml). After crystallization of the salts, the slurry was cooled to room temperature, stirred for 12 hours, then cooled to 4° C., and stirred for an additional 12 hours. The product was filtered, washed with cold ethyl acetate/hexane (2×5 ml) and cold hexane (2×5 ml), and dried in vacuo to give 8 g of the desired product.

c)



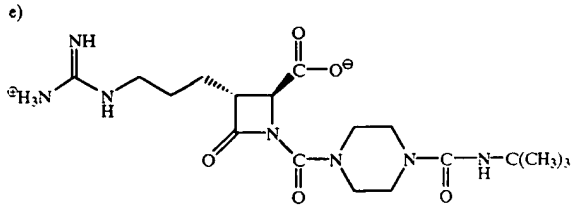
A dry, argon atmosphere 50 ml flask was charged with the product from part (b) (5.0 g). N,N'-Dimethylpropyleneurea (15 ml) was added, and the mixture was stirred for 5 minutes. The system was not homogeneous at this time. A 22° C. water bath was applied to the flask, and benzyl bromide (2.1 ml, 1.96 equivalents) was added rapidly (no exotherm was observed). tert-Butylamine (0.90 ml, 0.95 equivalents) was added dropwise (the temperature rose to 27.5° C. during the addition, held there for approximately 1 minute after the addition was complete, and then began to fall). When the temperature dropped to 25° C., the water bath was removed and the reaction was stirred overnight (16 hours). Completion of the reaction was confirmed by HPLC analysis. The reaction was diluted with ethyl acetate (30 ml) and tert-butyl methyl ether (30 ml). This solution was washed three times with 5% citric acid (1×30 ml, 2×15 ml) and then washed with saturated sodium chloride (1×15 ml). The resulting solution was dried over magnesium sulfate, filtered and concentrated in vacuo to give 5.85 g of the desired product as an orange oil.

d)



A dry, argon atmopsphere 50 ml flask was charged with the product from part (c) (2.5 g), anhydrous methylene chloride (25 ml), the carbamoyl chloride product from Example 32(c) or 193 (g) (1.3 g, 1.2 equivalents), triethylamine (0.98 ml, 1.6 equivalents), and 4-dimethylaminopyridine (0.16 g, 0.3 equivalents). The reaction was stirred for 5 hours. HPLC analysis confirmed that the reaction was 99.4% complete. The reaction mixture was shaken with 10% citric acid (aqueous, 25 ml). The aqueous layer was separated and extracted with methylene chloride (10 ml). The combined organic layers were washed with saturated sodium bicarbonate (aqueous, 25 ml) and with saturated sodium chloride (aqueous, 25 ml). The organic layer was separated, dried with magnesium sulfate, filtered and concentrated in vacuo to afford 3.46 g of the desired product as a tan foam.

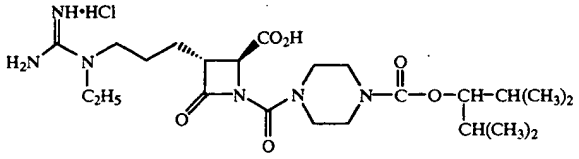
e)



A 12 L three necked round bottom flask was charged with 10% palladium on carbon catalyst (50.34 g, 47.31 mmol), water (362 ml), ethanol (6883 ml), and the product from part (d) (345 g). The mixture was agitated and sparged with nitrogen for approximately 20 to 30 minutes, then continuously sparged with hydrogen gas at 15° to 25° C. until HPLC analysis confirmed completion of the reaction. The reaction mixture was sparged with nitrogen for approximately 20–30 minutes, filtered, and the filter was washed 2L ethanol/water (95/5). The ethanol/water was partially concentrated in vacuo at room temperature to a solution of 8 to 10 ml per gram of product. Concentration gave a cloudy to white solution.

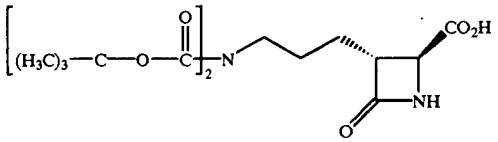
The above solution was allowed to crystallize overnight at room temperature with agitation (120–200 revolutions per minute). The product was filtered and the filter cake was washed three times with 300 ml of cold ethanol/water (95/5, 0° to 5° C.). The filter cake was dried in vacuo for 10 to 20 minutes. The resulting solid was dried to constant weight in a vacuum oven at room temperature to give 155 g of the desired final product as a white solid.

EXAMPLE 195



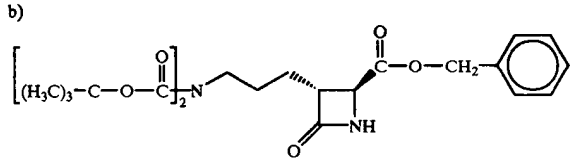
(homochiral)

a)



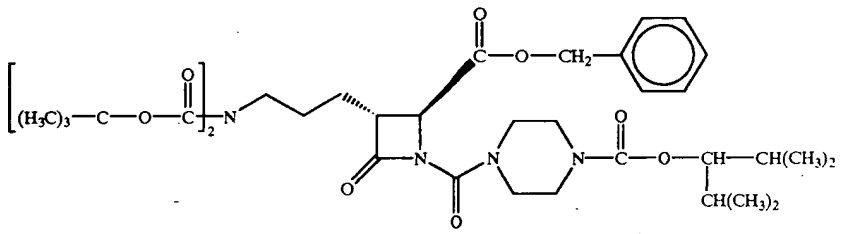
A. 2.5 M hexane solution of n-butyl lithium (5.75 ml, 14.39 mmol) was added dropwise to a stirred solution of diisopropylamine (2.02 ml, 14.39 mmol) in tetrahydrofuran (18ml) at 0° C. After 30 minutes of stirring the solution was cooled to -300C and a solution of (4S)-N-(t-butyldimethylsilyl)azetidinone carboxylic acid (1.50 g, 6.54 mmol) in tetrahydrofuran (8.0 ml) was added dropwise. The reaction mixture was stirred between -20° C. and -25° C. for 30 minutes. A solution of the iodo product from Example 192 step (b) (3.02 g, 7.85 mmol) in tetrahydrofuran (4.0 ml) was then added over 10 minutes. After 2 hours, the reaction mixture was warmed to 0° C. and quenched by the addition of ice cold water (35 ml). The pH was adjusted to 12.5 using 10% potassium bisulfate. After 30 minutes stirring, the solution was washed with hexanes, cooled to 0° C., and acidified to pH of 3.0 using 5N HCl. The aqueous solution was saturated with sodium chloride and extracted with ethyl acetate (twice). The organic extracts were combined, washed with brine, dried over magnesium sulfate, and concentrated to give 1.10 g of the desired product.

b)



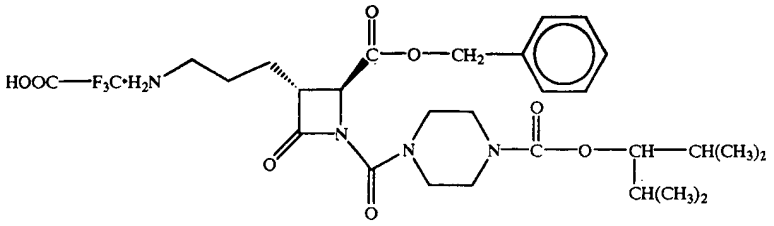
Sodium bicarbonate (0.62 g, 7.40 mmol) was added to a stirred solution of the product from step (a) (1.10 g, 2.96 mmol) in dimethylformamide (10 ml). Benzyl bromide (1.76 ml, 14.78 mmol) was then added. After 48 hours the reaction mixture was partitioned between ethyl acetate and water. The organic phase was isolated, washed with brine, dried over magnesium sulfate, and concentrated. The crude product was purified by silica gel chromatography to give 1.26 g of the desired product.

c)



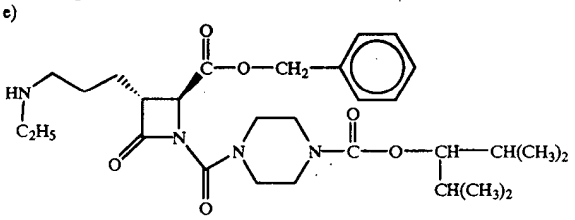
The benzyl ester product from step (b) (227 mg, 0.491 mmol) and 1-diisopropylmethoxycarbonylpiperazine-4-carbonylchloride (178 mg, 0.614 mmol) were dissolved in methylene chloride (2.0 ml). Triethylamine (103 μl, 0.737 mmol) was added followed by dimethylaminopyridine (6.0 mg, 0.049 mmol). After 48 hours the reaction mixture was concentrated and the residue was partitioned between ethyl acetate and water. The organic phase was isolated, washed with 5% potassium bisulfate and saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel chromatography to afford 300 mg of the desired product.

d)



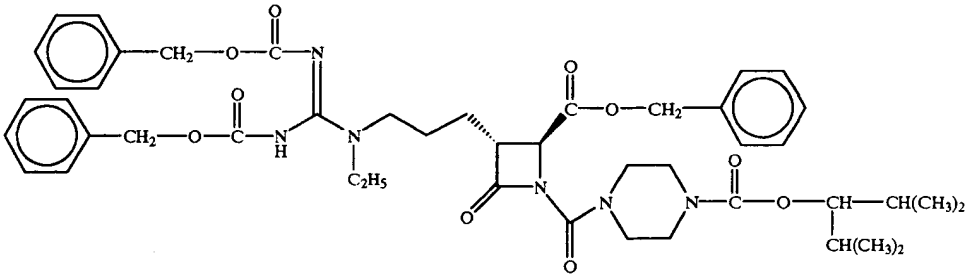
Trifluoroacetic acid was added dropwise to a stirred solution of the product from part (c) (300 mg, 0.418 mmol) in methylene chloride (1.8 ml) at 0° C. The reaction mixture was then stirred at room temperature. After one hour, the reaction mixture was concentrated and dried in vacuo. The crude product was dissolved in chloroform and concentrated. The procedure was repeated 3 times. The crude product was then dried in vacuo to give 297 mg of the desired product.

e)



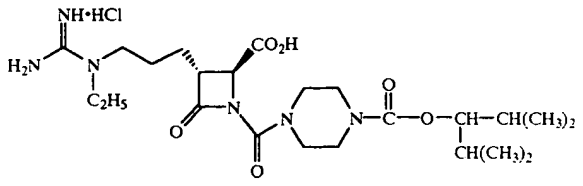
Acetaldehyde (15.0 mg, 0.330 mmol) was added to a stirred solution of the product from part (d) (198 mg, 0.314 mmol) in methylene chloride (1.50 ml). Acetic acid (36 μl, 0.628 mmol) was added. After 20 minutes of stirring, triacetoxy sodium borohydride (100 mg, 0.471 mmol) was added. After 24 hours the reaction mixture was diluted with ethyl acetate. One drop of acetic acid was added and the reaction was quenched by the addition of water. The organic phase was isolated, washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated to give 175 mg of the desired product.

f)



The product from part (e) (174 mg, 0.264 mmol) and N,N'-dicarbobenzyloxy—S-methyisothiourea (95 mg, 0.264 mmol) were dissolved in dimethylformamide (1.5 ml). Mercuric chloride (72.0 mg, 0.264 mmol) was added followed by triethylamine (110 μl, 0.792 mmol). After 3 hours, the reaction was diluted with ethyl acetate and filtered to remove mercury salts. The filtrate was washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The crude product was purified by silica gel chromatography to afford 45 mg of the desired product.

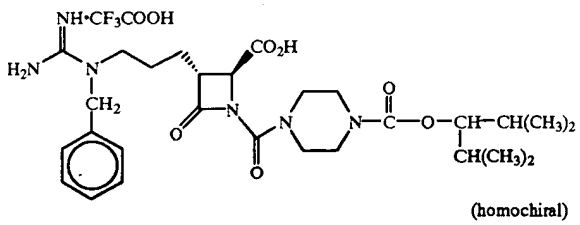
g)



(homochiral)

The product from part (f) (45 mg, 0.053 mmol) was dissolved in 1,4-dioxane (0.5 ml). 1N HCl (53 μl, 0.053 mmol) was added followed by 10% palladium on carbon catalyst (15 mg). A hydrogen atmosphere was introduced via balloon. After 3 hours of stirring at room temperature the reaction mixture was diluted with water: 1,4-dioxane (1:1) and filtered. The filtrate was lyophilized to afford 25 mg of the desired product; $(M+H)^+$=497.

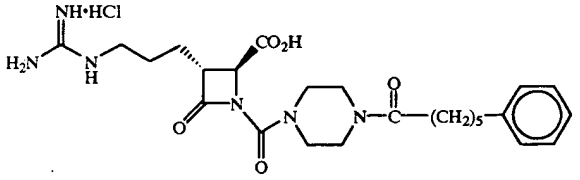
EXAMPLE 196



(homochiral)

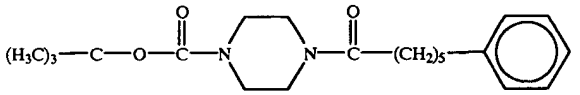
Following the procedure of Example 195 but employing benzaldehyde in place of acetaldehyde in step (e) the above compound was obtained and isolated as the trifluoroacetic acid salt; $(M+H)^+$=559.

EXAMPLE 197



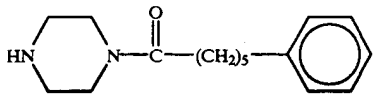
(homochiral)

a)



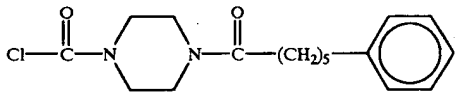
To a solution of 6-phenylhexanoic acid (4.0 g, 20.81 mmol) and hydroxybenztriazole (3.50 g, 22.89 mmol) in anhydrous methylene chloride (100 ml) was added ethyl-3—(dimethylamino)propyl carbodiimide, hydrochloride salt (4.39 g, 22.89 mmol) at 0° C. The mixture was stirred for 30 minutes. 1-tert-Butoxycarbonylpiperazine (3.88 g, 20.81 mmol) and diisopropylethylamine (4.35 ml, 24.97 mmol) were added and the mixture became a homogeneous solution. The solution was slowly warmed to room temperature over 3 hours and stirred overnight. The solvent was replaced with ethyl acetate (300 ml). The resulting solution was washed with 0.25 M potassium bisulfate (pH of 3 to 4), saturated sodium bicarbonate (pH of 9 to 10), and brine, dried over magnesium sulfate, and concentrated to give the desired product in crude form as a colorless oil.

b)



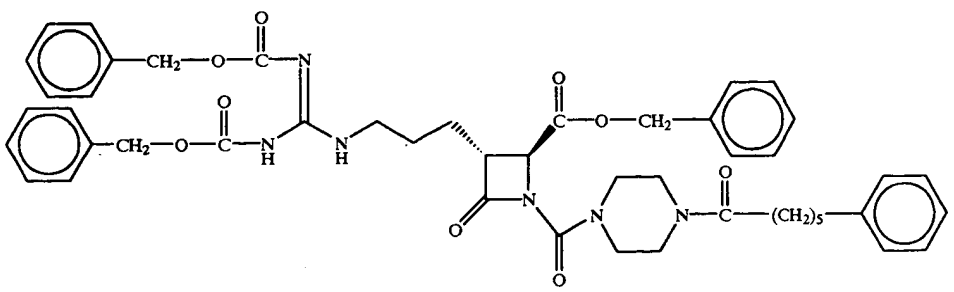
The crude product from part (a) was dissolved in methylene chloride (160 ml). The solution was cooled to 0° C. and trifluoroacetic acid (40 ml) was added dropwise. The ice-bath was removed. The mixture was stirred at room temperature for one hour. The solvents were removed under vacuum. The residue was diluted with ethyl acetate (200 ml). The solution was neutralized with saturated sodium bicarbonate (pH of 10). The aqueous layer was extracted with ethyl acetate (2×100 ml). The combined ethyl acetate solution was washed with brine, dried over magnesium sulfate and concentrated to give the desired product as a colorless oil.

c)



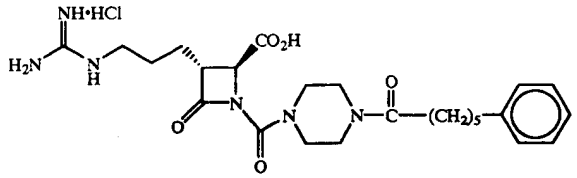
A solution of phosgene (20% in toluene, 50 mmol, 26.3 ml) was dissolved in methylene chloride (30 ml) and cooled to 0° C. A solution of the crude product from part (1)) and triethylamine in methylene chloride (30 ml) was slowly added to the above solution over 20 minutes. The resulting solution was stirred at 0° C. for 1.5 hours. The precipitate was removed by filtration. The filtrate was concentrated and the residue was purified with silica gel chromatography (hexane:ethyl acetate, 2:1, R_f=0.15) to afford 5.00 g of the desired product as a white solid; $(M+H)^+$=323.3; IR (KBr) 1731 cm^{-1}.

d)



To a solution of the benzyl ester product from Example 1(c) (150 mg, 0.26 mmol) in methylene chloride (3 ml) was added triethylamine (0.043 ml, 0.31 mmol), the product from part (c) (102 mg, 0.31 mmol), and 4-dimethylaminopyridine (1.6 mg, 0.015 mmol). The solution was stirred for 3 hours and the solvent was removed. The residue was purified with silica gel chromatography (hexane:ethyl acetate, 1:1, R_f=0.22) to afford 210 mg of the desired product as a colorless oil. $(M+H)^+$=859.5; $(M-H)^-$=857.5.

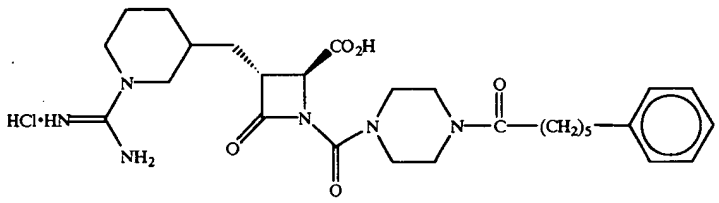
e)



(homochiral)

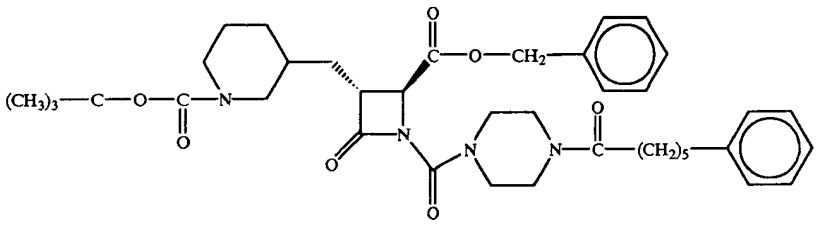
A mixture of the product from part (d) (100 mg, 0.115 mmol), palladium on carbon catalyst (10%, 30 mg), and 1N HCl (115 μl, 0.115 mmol) was stirred under a hydrogen atmosphere (balloon) at room temperature for 45 minutes. Analytical HPLC indicated the reaction was completed. The reaction mixture was diluted with water (6 ml), filtered, and lyophilized to give 53 mg of the desired product as a white powder. $(M+H)^+$=501.3;; $(M-H)^-$=499.2; IR (KBr) 1785 cm^{-1}.

EXAMPLE 198



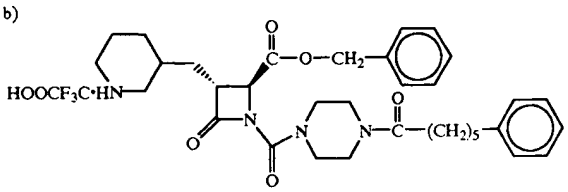
(diastereomeric mixture)

a)



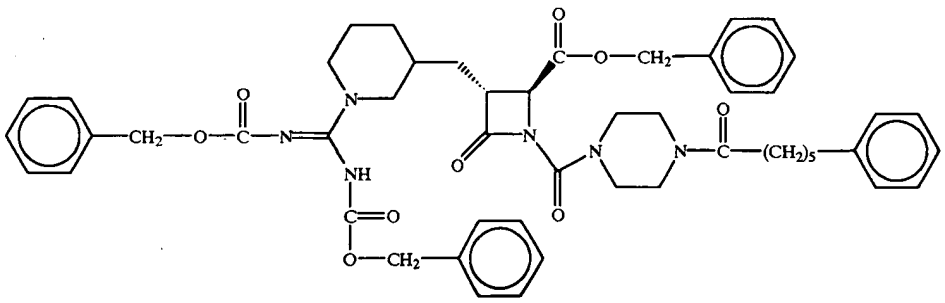
A solution of the product from Example 172(f) (80.5 mg, 0.20 mmol), triethylamine (0.056 ml, 0.40 mmol), the product of Example 197 (c) (97 mg, 0.30 mmol) and dimethylaminopyridine (6 mg, 0.05 mmol) in methylene chloride (1 ml) was stirred at room temperature under argon for 21 hours. The reaction was concentrated in vacuo and the residue was taken up in ethyl acetate, 10% potassium bisulfate, and water. The ethyl aceate layer was washed again with dilute potassium bisulfate, water (2×) and brine, dried over sodium sulfate and concentrated to an oil (179 mg). Chromatography of the oil over silica gel using 15% and then 25% ethyl acetate in methylene chloride provided 122 mg of the desired product as an oil.

b)



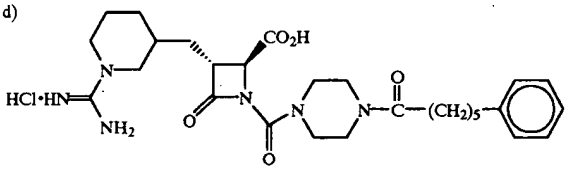
Treatment of the product from part (a) (120 mg, 0.174 mmol) with trifluoroacetic acid in methylene chloride according to the procedure described in Example 172 step (h) afforded 174 mg of the crude desired product.

c)



Treatment of the crude product from part (b) with N,N'-dicarbobenzyloxy-S-methylisothiourea according to the procedure in Example 172 step (i) gave 204 mg of crude product. Purification by chromatography over silica gel using methylene chloride: ethyl acetate (3:1) gave 79 mg of the desired product as an oily residue.

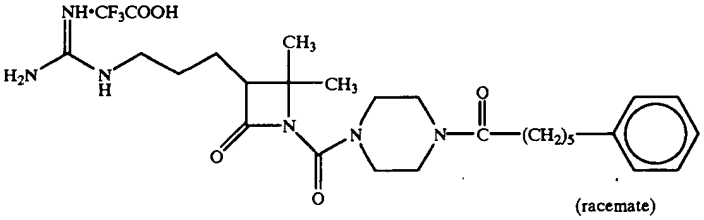
d)



(diastereomeric mixture)

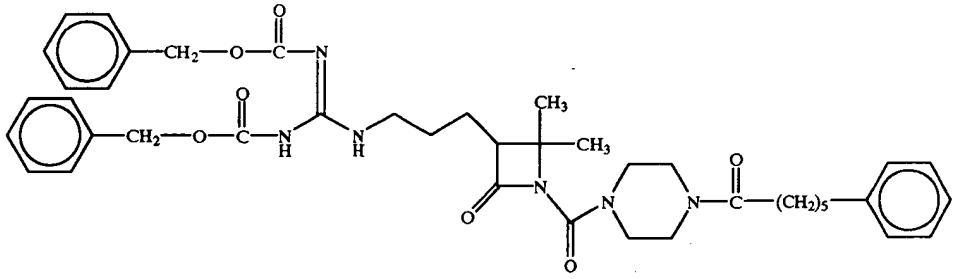
The product from step (c) (76 mg, 0.085 mmol) was hydrogenated in dioxane (3 ml) and 1.0N HCl (0.085 ml, 0.085 mmol) in the presence of 10% palladium on carbon catalyst (24 mg) at 1 atmosphere of hydrogen for 1 hour. After filtration using aqueous dioxane, the filtrate was concentrated to remove dioxane, filtered and lyophilized to give 42 mg of the desired proudct as a white solid; (IR (KBr) 1784 cm^{-1}, consisting of a mixture (62.38) of diastereomers as determined by HPLC.

EXAMPLE 199



(racemate)

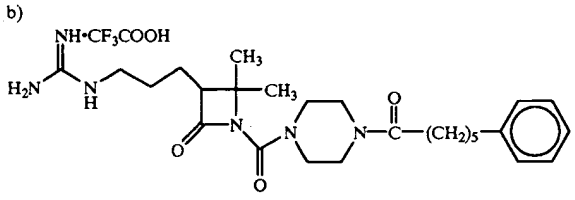
a)



To a stirred solution of the protected dimethyl azetidinone from Example 135(f) (55 mg, 0.12 mmol) in tetrahydrofuran (3 ml) at −78° C. was added sodium bis(trimethylsilyl)amide (1N in hexanes, 0.13 mmol) and the solution was kept at this temperature for 30 minutes. A solution of the product from Example 197(c) (43 mg, 0.13 mmol) in tetrahydrofuran (1

ml) was added dropwise and the resulting mixture was warmed to -20° C. After 1 hour, the reaction was quenched with aqueous saturated ammonium chloride (5 ml) and extracted with ethyl acetate (2x5 ml). The combined organic layers were washed with brine and dried over magnesium sulfate. Filtration, concentration and purification by column chromatography (silica gel, 50% ethyl acetate in hexanes) afforded 79 mg of the desired product as a colorless oil.

b)

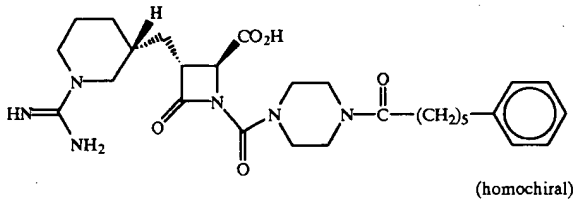


(racemate)

To a stirred solution of the product from part (a) (79 mg, 0.11 mmol) in a mixture of ethanol (1.5 ml), water (0.5 ml and ethyl acetate (1.5 ml) at room temperature was added palladium on carbon catalyst (10% wet, 15 mg). The resulting suspension was bubbled with hydrogen for 3 hours and the reaction mixture was then filtered. Concentration and purification by preparative HPLC (YMC ODS A 20x250 mm, 5 μ, 0 to 100% B over 35 minutes, hold time 15 minutes, A=10% methanol in water and 0.1% trifluoroacetic acid, B=90% methanol in water and 0.1% trifluoroacetic acid) afforded 42 mg of the desired product as a white solid; $(M+H)^+$=485.

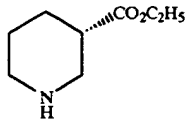
EXAMPLE 200

(2S, 3R)-3-[[(3R)-1-(Aminoiminomethyl)-3-piperidinyl]methyl]-4-oxo-1-[[4-(1-oxo-6-phenylhexyl)-1-piperazinyl]carbonyl]-2-azetidinecarboxylic acid



(homochiral)

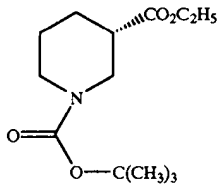
a)



(±) Ethylnipeconate (100 g) and D-(-)tartaric acid (96 g) were dissolved in ethanol (500 ml, 95%) and heated to give a solution, which was filtered to remove minor insoluble material. The flask containing the solution was placed in a hot water bath to allow a very slow cooling and crystallization. After 4 hours, the formed crystals were filtered off and washed with ice cold ethanol (50 ml, 100%) yielding 70 g of the S(-) tartaric acid salt of ethylnipeconate. The mother liquor was concentrated and set aside. The crystallization was repeated twice to give 58 g of the S(-) tartaric acid salt. $[\alpha]_D$=-10.56° (c=5% in water). See. R. Gollamudi et al., Med. Chem. Res., Vol 4, p. 597-603 (1994); $[\alpha]_D$=-10.50° (c=5% in water).

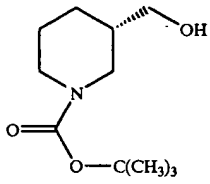
The free base was liberated from an ice cold aqueous solution of the 32 g of the S(-) tartrate salt at pH 11.5 (3.0 N sodium hydroxide solution) by extraction with chloroform. Concentration of the dried chloroform solution gave 12.9 g of the desired product as a colorless mobile oil. $[\alpha]_D$=+1.69 (c=5% in water).

b)



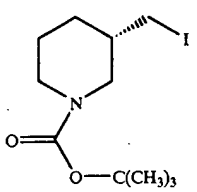
The product from part (a) (10 g, 63.61 mmol) was dissolved in dry ether (400 ml) at 0° C. with stirring and a solution of di-t-butyldicarbonate (13.9 g) in ether (20 ml) was added over 10 minutes. After stirring overnight at room temperature, the reaction mixture was cooled to 0° C., placed in an ice bath, and citric acid solution (50 ml, 35%) was slowly added with stirring. After phase separation, the ether layer was washed with brine (3x300 ml) and dried over sodium sulfate. Concentration gave 13.8 g of the desired product as a colorless oil.

c)



A solution of 1.0 N lithium aluminum hydride in tetrahydrofuran (52.4 ml) was added to the product from part (b) (13.5 g, 52.4 mmol) dissolved in tetrahydrofuran (100 ml) at 0° C. over a period of 15 minutes. The reaction was completed after 1.5 hours with stirring. The slow addition of 5.0 N sodium hydroxide (6.8 ml) with stirring, filtration, and concentration of the filtrate yielded an oil. This oil was dissolved in ether (80 ml). The ether solution was washed with brine and concentrated to a colorless oil, which on standing crystallized to give 10.18 g of the desired product as white crystals.

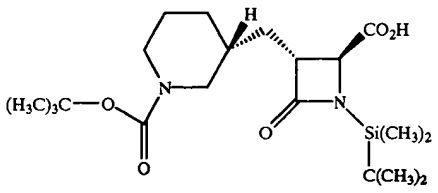
d)



A solution of the product from part (c) (9.0 g, 41.8 mmol) in methylene chloride (50 ml) was added dropwise to a solution triphenylphosphine (16.13 g, 61.5 mmol), imidazole (4.19 g, 61.5 mmol) and iodine (15.6 g, 61.5 mmol) in methylene chloride (120 ml) at 0° C. (addition takes approximately 15 minutes). After stirring for 1.5 hours at room temperature, the reaction mixture was filtered and the filtrate was concentrated and taken up in ethyl acetate (100

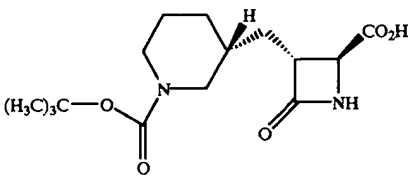
ml). The resulting solution was washed with 5% sodium thiosulfate solution until the yellow color disappeared, washed with brine, dried over magnesium sulfate, and concentrated to an oil which crystallized slowly on standing. The material was purified by column chromatography on 300 g silica gel (methylene chloride/hexane, 4:1) and a second column chromatography on 200 g silica gel (ethyl acetate/hexane, 2:3) to give 11.1 g of desired product as white crystals. $[\alpha]_D$=+8.38° (c=5% in chloroform).

e)



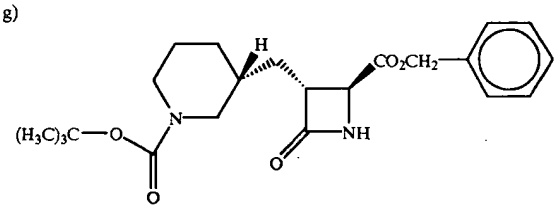
To a solution of diisopropylamine (7.83 ml, 52.7 mmol) in dry tetrahydrofuran (50 ml) was added at −20° C. a solution of 2.5 N butyl lithium in hexane (18.64 ml, 46.6 mmol). After stirring at −20° C. for 30 minutes, the solution was cooled to −70° C. and a solution of (4S)-N-(t-butyldimethylsilyl)azetidine-2-one-4-carboxylic acid (5.36 g, 46.6 mmol) in tetrahydrofuran (15 ml) was slowly added. After the addition was completed, the temperature was allowed to rise to −20° C. and a solution of the product from step (d) (10.5 g, 32.3 mmol) was added and the reaction mixture was stirred at −20° C. for 30 hours. The resulting suspension was poured into 200 ml ice/water and the pH was adjusted to 2.0. After extraction with ethyl acetate (five times), the ethyl acetate extracts were combined, washed with brine, and concentrated to an oil. This oil was dissolved in ether (100 ml) and the ether was extracted (five times) with 30 ml of sodium bicarbonate solution (5%). The combined aqueous layer at 0° C. was acidified with potassium bisulfate solution to pH 2.0 and extracted with ethyl acetate (3×30 ml). The organic layer was washed with brine, dried, and concentrated to give 7.82 of the desired product as an off-white foam.

f)



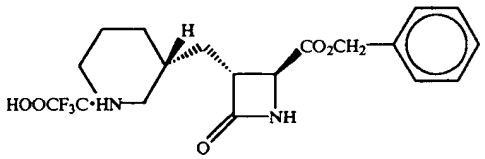
A solution of the product from step (e) (7.8 g) and 22.8 ml of tetrabutyl ammonium fluoride (1.0 N in tetrahydrofuran) was stirred for 30 hours at 0° C. and for one hour at room temperature. After concentration, the residue was dissolved in ethyl acetate (50 ml) and poured into 100 ml ice/water. The pH was then adjusted to 2.0 with potassium bisulfate solution, the phases were separated, and the aqueous layer was extracted with ethyl acetate (4×30 ml). The organic layers were combined, concentrated, and dried to yield 5.32 g of the desired product.

g)



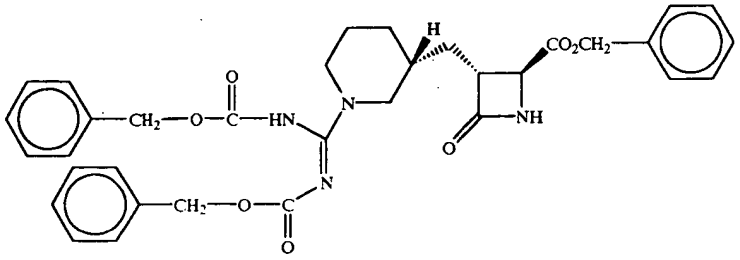
The product from part (f) (5.3 g, 12.88 mmol), benzyl bromide (6.3 ml, 53.0 mmol), and sodium bicarbonate (4.45 g, 53.0 mmol) were stirred at room temperature for 24 hours in dimethylformamide (60 ml). After 60 ml of ice/water was added, the mixture was extracted with ethyl acetate (4×30 ml). The combined organic layer was washed with brine and dried over sodium sulfate. Concentration gave a crude colorless oil which was purified by flash column chromatography on 300 g of silica gel (ethyl acetate/hexane, 1:1). The resulting colorless oil crystallized by scratching with a small amount of ether to give 5.8 g of the desired product as white crystals. MS $(M+H)^+$=403.

h)



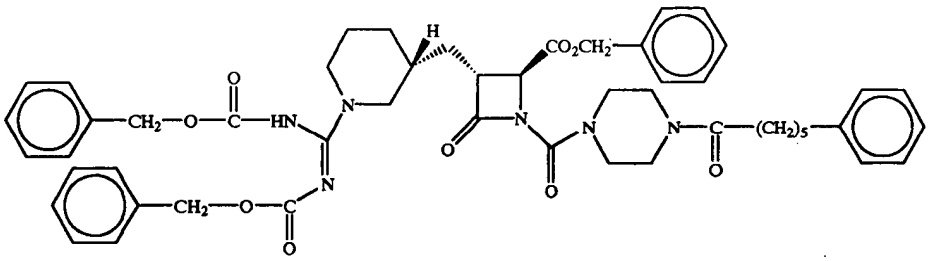
The product from part (g) (5.7 g, 14.18 mmol) was dissolved in methylene chloride (25 ml) and trifluoroacetic acid (10 ml) was added dropwise with stirring. The reaction was completed in one hour. The reaction mixture was concentrated and evaporated (3 times) from toluene (10 ml) to remove excess trifluoroacetic acid and give 7.96 g of the desired product as a white foam.

i)



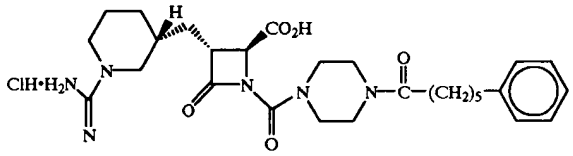
The product from part (h) (7.95 g), N,N'-bis (benzyloxycarbonyl)-1-guanylpyrazole (7.8 g, 21.4 mmol) and triethylamine (12.9 ml, 92.5 mmol) were stirred in dimethylformamide (50 ml) at 0° C. for one hour and for an additional 24 hours at room temperature. Ice water (50 ml) was added and the pH was adjusted to 3.0 with potassium bisulfate solution. The aqueous layer was extracted with ethyl acetate (5×30 ml). The organic layers were combined and washed with 5% potassium bisulfate solution, which was saturated with sodium chloride, half saturated brine, and brine. After drying and concentration, 12.05 g of crude product was obtained as a yellow-brown oil. Purification by column chromatography on silica gel (methylene chloride/ethyl acetate, 8:2 and 7.3) gave 6.9 g of product as a colorless oil. This oil crystallized when stirred with ether and seed crystals (obtained from a small amount of oil in diisopropyl ether/pentane) to give 5.6 g of the desired product as white crytals. IR(KBr) 1749 cm^{-1}; MS $(M+H)^+$= 613.

j)



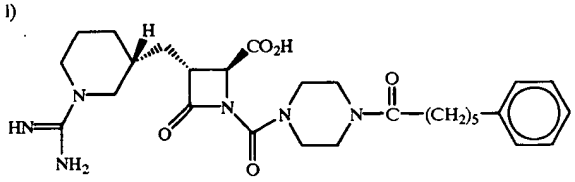
The product from part (i) (800 mg, 1.30 mmol), the product from Example 197(c) (421 mg, 1.31 mmol), diisopropylethylamine (343 μl) and 10 mg of catalytic dimethylaminopyridine were dissolved in 8 ml of methylene chloride and stirred for 24 hours at room temperature. After concentration, the reaction mixture was taken up with ethyl acetate (20 ml), extracted with potassium bisulfate/ice water solution at pH 3.0, washed with brine, and dried over sodium sulfate to yield from the organic phase 1.08 g of crude product. Purification by flash chromtography on 200 g silica gel (ethyl acetate/hexane, 3:2) gave 746 mg of desired product as a white foam. IR (film) 1786 cm^{-1}; MS $(M+H)^+$= 899.

k)



The product from part (j) (740 mg, 0.82 mmol) was dissolved in dioxane (10 ml). Palladium on carbon catalyst (400 mg, 10%) and 1.0 N HCl (823 μl) were added and the mixture was hydrogenated (balloon) for 2.5 hours. Filtration, concentration of the filtrate, and lyophilization yielded 465 mg of the desired product as a white powder. IR (KBr) 1785 cm^{-1}; MS$(M+H)^+$=541.

l)



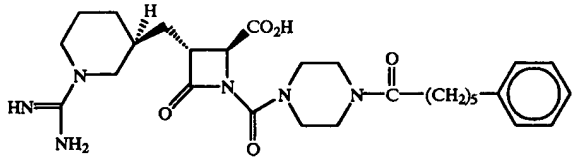
The product from part (j) (455 mg, 0.79 mmol) was dissolved in aqueous dioxane and passed through a column of 5 g of polyvinylpyridine packed in water-dioxane (70:30).

Product containing fractions were combined, concentrated, and lyophilized to give 375 mg of the desired product as a white solid. IR (KBr) 1775 cm^{-1}; MS $(M+H)^+$=541.

Anal. calc'd for $C_{28}H_{40}N_6O_5.1.60\ H_2O$: C, 59.05; H, 7.65; N,14.76; H_2O, 5.06. Found: C,58.81; H, 7.60; N. 14.12; H_2O, 4.61.

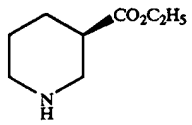
EXAMPLE 201

(2S,3R)-3-[[(3S)-(Aminoiminomethyl)-3-piperidinyl]methyl]-4-oxo-1-[[4-(1-oxo-6-phenylhexyl)-1-piperazinyl]carbonyl]-2-azetidinecarboxylic acid



(homochiral)

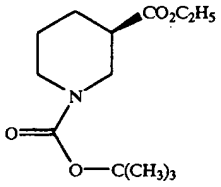
a)



Following the procedure of Example 200 step (a) but employing L-(+) tartaric acid for the resolution, the R(+) tartaric acid salt of ethylnipeconate was obtained. $[\alpha]_D$=+ 10.68° (c=5% in water). See R. Gollamudi et al, Med. Chem. Res., Vol. 4, p. 597–603; $[\alpha]_D$=10.60° (c=5% in water).

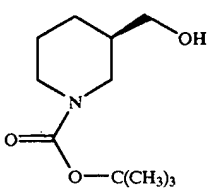
The free base was liberated from the R(+) tartaric acid salt as a colorless liquid; $[\alpha]_D$=−1.68° (c=5% in water).

b)



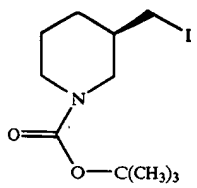
The product from part (a) was reacted with di-t-butyldicarbonate according to the procedure of Example 200(b) to give the desired product as a colorless oil that crystallized after standing.

c)



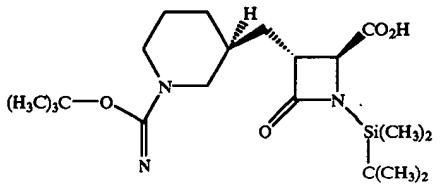
The product from part (b) was reacted with lithium aluminum hydride according to the procedure of Example 200(c) to give the desired product as white crystals.

d)



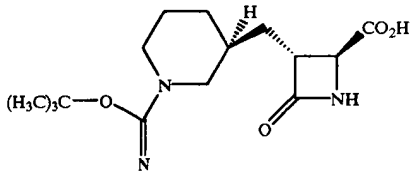
The product from part (c) was reacted with triphenylphosphine, imidazole and iodine according to the procedure of Example 200 (d) to give the desired product as white crystals. $[\alpha]_D$=−8.42° (c=5% in chloroform).

e)



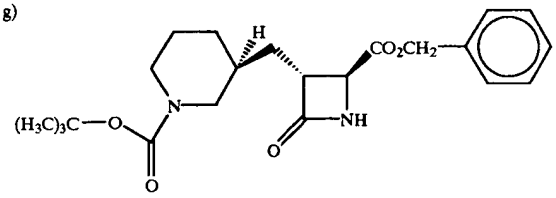
The product from part (d) was reacted with (4S)-N-(t-butyldimethylsilyl)azetidine-2-one-4-carboxylic acid according to the procedure of Example 200(e) to give the desired product as a yellow foam.

f)



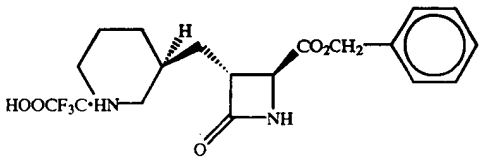
The product from part (e) was reacted with tetrabutyl ammonium fluoride according to the procedure of Example 200(f) to give the desired product as a somewhat yellow foam.

g)



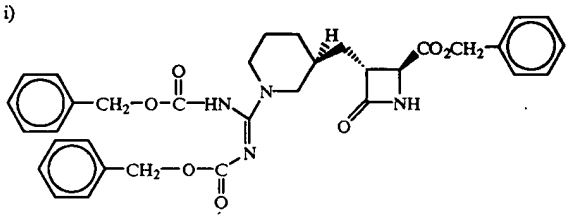
The product from part (f) was reacted with benzyl bromide according to the procedure of Example 200(g) to give the desired product as white crystals from ethyl acetate (the product did not crystallize directly from the concentrated work-up solution). IR (KBr) 1770 cm^{-1}; MS $(M+H)^+$=403.

h)



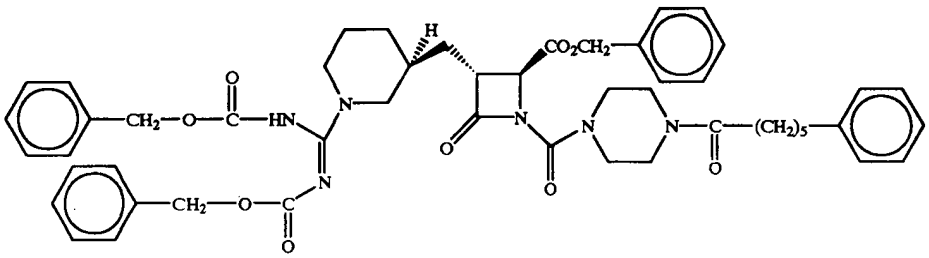
The product from part (g) was reacted with trifluoroacetic acid according to the procedure of Example 200(h), to give the desired product as an off-white foam.

i)



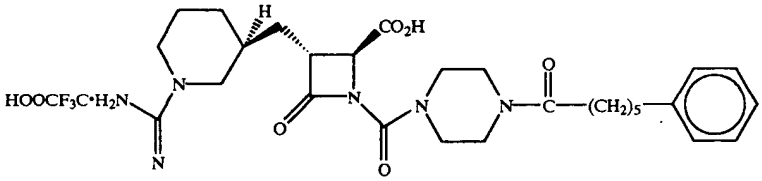
The product from part (h) was reacted with N,N'-bis (benzyloxycarbonyl)-1-guanylpyrazole according to the procedure of Example 200(i) to give 4.8 g of crude product that crystallized directly from the work-up concentrated ethyl acette solution. Recrystallization from methylene chloride/hexane gave the desired product as off-white crystals. IR (KBr) 1753 cm^{-1}; MS $(M+H)^+$=613.

j)



The product from part (i) was reacted with the product from Example 197(c) according to the procedure of Example 200(j) to give the desired product as a white foam. IR (film) 1785 cm^{-1}; MS $(M+H)^+$=899.

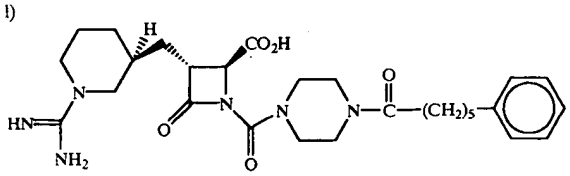
k)



The product from part (j) was hydrogenated according to the procedure of Example 200(k) to give the product as an HCl salt. IR (KBr) 1784 cm^{-1}; MS $(M+H)^+$=514.

The above trifluoroacetic acid salt was obtained by preparative HPLC (reverse phase methanol, water, trifluoroacetic acid) to give the desired product as a white lyophilate.

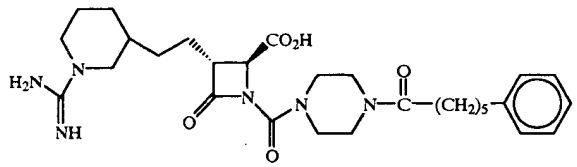
l)



The product from part (k) was dissolved in aqueous dioxane and passed through a column of 5 g of polyvinylpyridine packed in water-dioxane (70:30). Product containing fractions were combined, concentrated and lyophilized to give the desired product as a white solid. IR (KBr) 1776 cm^{-1}; MS $(M+H)^+$=541.

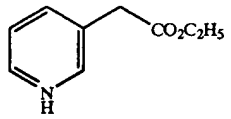
Anal. calc'd for $C_{28}H_{40}N_6O_{5-1.30}H_2O$: C, 59.62; H, 7.61; N, 14.90; H_2O, 4.15. Found: C, 59.62; H, 7.55; N, 14.65; H_2O, 3.95.

EXAMPLE 202



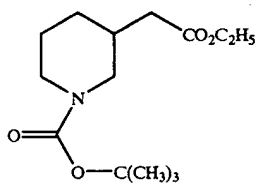
(diastereomeric mixture)

a)



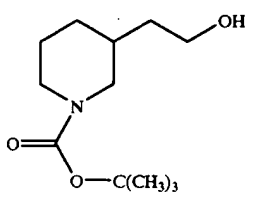
Ethyl 3-pyridylacetate (13.2 g, 80 mmol) was hydrogenated in 40 ml of absolute ethanol and 40 ml of acetic acid with 0.8 g of platinum oxide at 3 atmospheres for 20 hours. After filtration and removal of solvents in vacuo, the yellow residual oil was dissolved in chloroform and treated with a solution of 45 g of potassium carbonate in 200 ml of water. After extraction with chloroform (3×), the chloroform extract was dried over sodium sulfate and concentrated to an oil. The oil was taken up in ether and then concentrated to give 11.73 g of the desired product as an oil.

b)



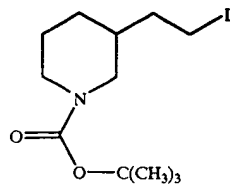
A solution of di-t-butyldicarbonate (15.0 g, 68.7 mmol) in ether (15 ml) was added over several minutes to a stirred solution of the product from part (a) (11.73 g, 68.5 mmol) in ether (100 ml) under nitrogen and stirred overnight at room temperature. Aqueous citric acid solution (50 ml of a solution of 350 g/1000 ml water) was added slowly to the reaction cooled in an ice water bath. The layers were separated and the aqueous layer was extracted with ether. The combined ether layers were washed with water, brine, and water, dried over sodium sulfate, and concentrated to give 17.95 g of the desired product as a yellow oil.

c)



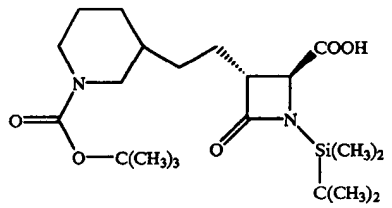
The product from part (b) was treated with lithium aluminum hydride according to the procedure of Example 200(c) to give the desired product as a colorless oil.

d)



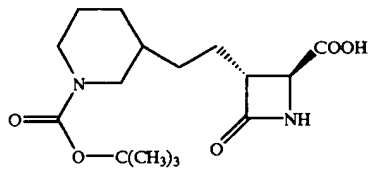
The product from part (c) was treated with triphenylphosphine, imidazole and iodine according to the procedure of Example 200(d) to give the desired product as an amorphous solid.

e)



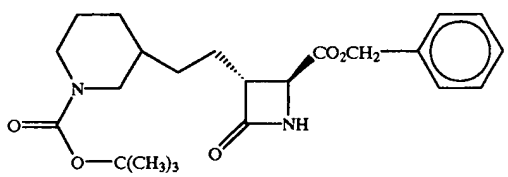
The product from part (d) was reacted with (4S)-N-(t-butyldimethylsilyl)azetidine-2-one-4-carboxylic acid according to the procedure of Example 200(e) to give the desired product.

f)



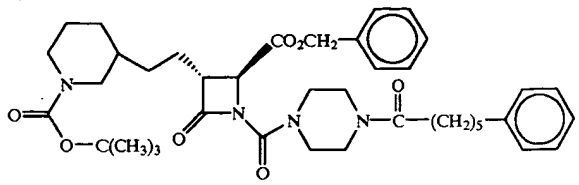
The product from part (e) was reacted with tetrabutyl ammonium fluoride according to the procedure of Example 200(f) to give the desired product as an oil.

g)



The product from part (f) was reacted with benzyl bromide according to the procedure of Example 200(g) to give the desired product as an amorphous white solid.

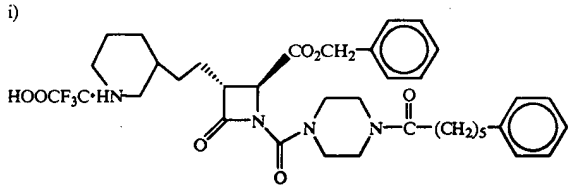
h)



A solution of the product from part (g) (167 mg, 0.40 mmol), triethylamine (0.116 ml, 0.80 mmol), the product from Example 197(c) (194 mg, 0.60 mmol) and dimethy-

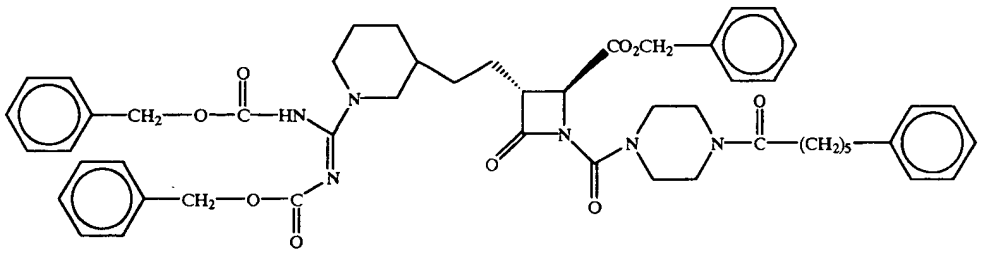
laminopyridine (12 mg, 0.10 mmol) in methylene chloride (2 ml) was stirred at room temperature under argon for 20 hours. The reaction was concentrated in vacuo and the residue was taken up in ethyl acetate, 10% potassium bisulfate and water. The ethyl acetate layer was washed again with dilute potassium bisulfate, water (2×), and brine, dried over sodium sulfate, and concentrated to give 362 mg of an oil. Chromatography of the oil over silica gel using 10%, 20% and the 35% ethyl acetate in methylene chloride gave 247 mg of the desired product as an oil.

i)



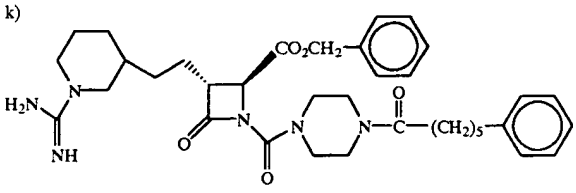
Trifluoroacetic acid (0.50 ml) was added dropwise to a stirred solution of the product from part (h) (170 mg, 0.242 mmol) in methylene chloride (1.50 ml) at 0° C. The reaction mixture was then stirred at room temperature. After one hour, the reaction mixture was concentrated and dried in vacuo. The crude product was dissolved in chloroform and concentrated. This procedure was repeated three times. The crude product was then dried in vacuo to give 173 mg of the desired product.

j)



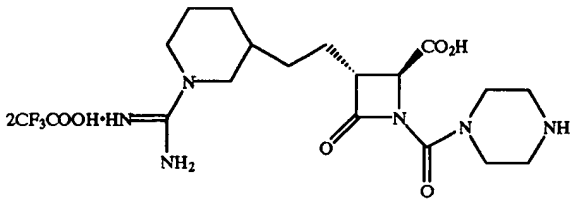
The product from part (i) (173 mg, 0.241 mmol) and N,N'-dicarbobenzyloxy-S-methylisothiourea (260 mg, 0.362 mmol) were dissolved in dimethylformamide (2.0 ml). Mercuric chloride (98.0 mg. 0.362 mmol) was added followed by triethylamine (134 μl, 0.964 mmol). After 2 hours the reaction mixture was diluted with ethyl acetate and filtered to remove mercury salts. The filtrate was washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The crude product was purified by silica gel chromatography to give 101 mg of the desired product.

k)



The product from part (j) (94 mg, 0.103 mmol) was dissolved in 1,4-dioxane (1.0 ml). 1N HCl (103 μl, 0.103 mmol) was added followed by 10% palladium on carbon catalyst (25 mg). A hydrogen atmosphere was introduced via balloon. After 2 hours of stirring at room temperature, the mixture was diluted with water (1.0 ml) and filtered. The solution was then put on a one inch column of polyvinylpyridine resin which had been cleaned by rinsing with methylene chloride, methanol and water. The column was eluted with water. Product containing fractions were combined and lyophilized to give 36.6 mg of the desired product. MS(M+H)$^+$ 555.

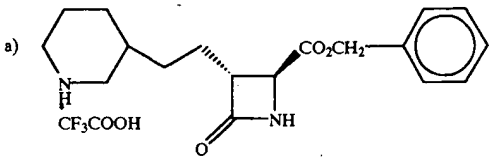
EXAMPLE 203



(diastereomeric mixture)

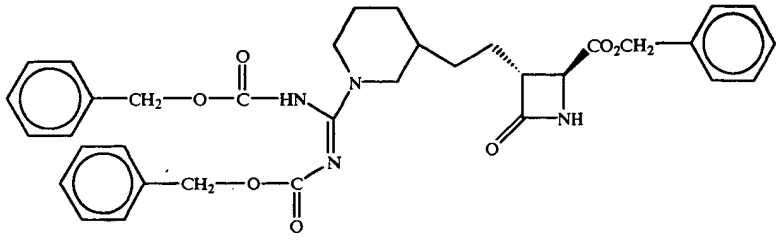
-continued

a)



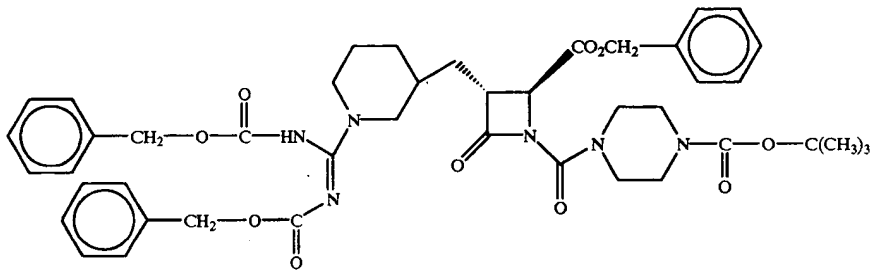
Treatment of the product of Example 172(f) (12.6 g, 31.3 mmol) with trifluoroacetic acid (25 ml) in methylene chloride (100 ml) according to the procedure of Example 172(h) afforded the desired product (crude) as a viscous oil.

b)



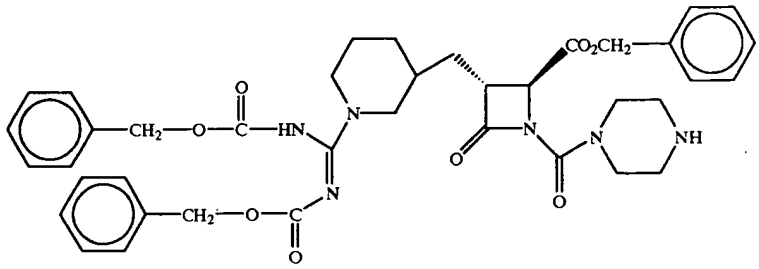
Triethylamine (22 ml, 158 mmol) was slowly added to a solution of the crude product from part (a) (31.3 mmol) and N,N'dicarbobenzyloxy-S-methylisothiourea (13.0 g) in dimethylformamide (31 ml) under argon cooled in an ice-water bath. The reaction was stirred at room temperature for 20 hours and concentrated to a residue. The residue was taken up in ethyl acetate (500 ml). The ethyl acetate was washed with water (500 ml) and the aqueous layer was extracted with ethyl acetate (200 ml). The combined ethyl acetate layer was washed with dilute potassium bisulfate plus brine (2×) and brine (2×), dried over sodium sulfate, and concentrated to a viscous oil (23 g). Chromatography of the oil over silica gel using methylene chloride-ethyl acetate (8:2) and then (1:1), followed by concentration of the combined fractions from ethyl acetate gave 14.1 g of the desired product as a white solid consisting of a mixture (60:40) of diastereomers.

c)



Triethylamine (90 μl, 0.66 mmol) and 4-dimethylaminopyridine (5 mg, 0.04 mmol) were added to a stirred solution of the product from part (b) (132 mg, 0.22 mmol) and the carbamoyl chloride from Example 20(a) (59 mg, 0.24 mmol) in methylene chloride (5 ml) at room temperature. After 2 hours, the reaction mixture was concentrated and purified by silica gel chromatography (50% ethyl acetate in hexanes) to give 177 mg of the desired product as a white solid.

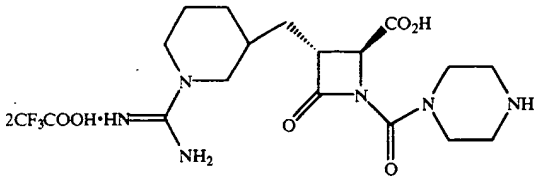
d)



Trifluoroacetic acid (0.2 ml, 2.46 mmol) was added to a stirred solution of the product from part (c) (177 mg, 0.22 mmol) in methylene chloride (5 ml) at room temperature. After 30 minutes, the reaction was first concentrated under reduced pressure and then diluted with ethyl acetate. Aqueous sodium bicarbonate was added to the resulting organic

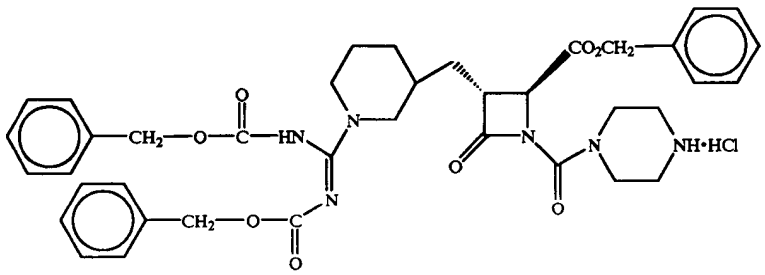
solution until the pH of the aqueous layer reached 10. The aqueous layer was extracted with ethyl acetate and the combined organic layers were dried over magnesium sulfate. Filtration and concentration under reduced pressure gave 125 mg of the desired product as a colorless oil.

e)



Palladium on carbon catalyst (10%, 20 mg) was added to a stirred solution of the product from part (d) (40 mg, 0.06 mmol) in a mixture of ethyl acetate (2 ml), ethanol (2 ml) and water (1 ml) at room temperature. Hydrogen gas was bubbled through the resulting suspension for 3 hours. After filtration through a plug of Celite® and concentration, the mixture was purified by preparative HPLC (YMC ODS A 20×250 mm, 5 μl, 0 to 100% B over 30 minutes, hold time 15 minutes, A=10% methanol in water and 0.1% trifluoroacetic acid, B=90% methanol in water and 0.1% trifluoroacetic acid) to afford 8 mg of the desired trifluoroacetic acid salt product as a white solid. MS $(M+H)^+$=367.

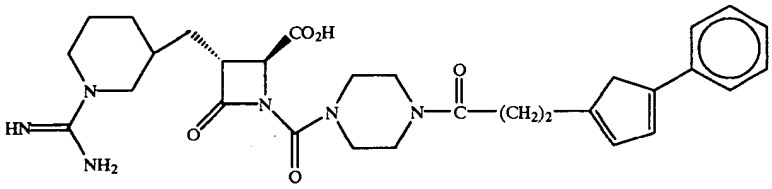
f)



The above intermediate was prepared as follows.

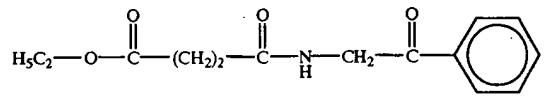
Trifluoroacetic acid (0.2 ml, 2.46 mmol) was added to a stirred solution of the product from part (c) (177 mg, 0.22 mmol) in methylene chloride (5 ml) at room temperature. After 30 minutes, the reaction was concentrated under reduced pressure. The resulting oil was diluted with methylene chloride followed by the addition of HCl in diethyl ether (1N, 0.30 ml, 0.30 mmol). Additional diethyl ether (3.0 ml) induced formation of a white precipitate which was filtered to give the desired HCl salt as a white solid.

EXAMPLE 204



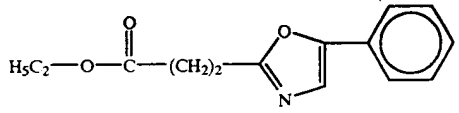
(diastereomeric mixture)

a)



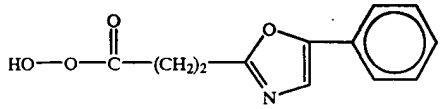
Ethyl succinylchloride (1.92 g, 11.65 mmol) was added to a solution of 2-aminoacetophenone (2.00 g, 11.65 mmol) in pyridine (12 ml) at 0° C. The mixture was stirred at room temperature for 20 hours and then diluted with chloroform (75 ml) and water (10 ml). The organic layer was then separated and washed with 5% sodium bicarbonate (20 ml), dried over magnesium sulfate, and concentrated to give 3.14 g of the desired product as an orange solid.

b)



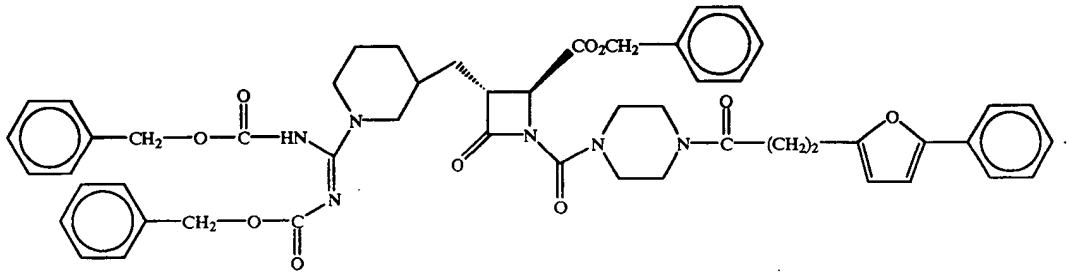
A mixture of the product from part (a) (2.1 g) and phosphorus pentoxide (4.53 g, 31.9 mmol) in chloroform (20 ml) under nitrogen was heated at reflux for 16 hours. The solution was cooled to room temperature and ice was added followed by neutralization with 5% sodium bicarbonate. The mixture was extracted with chloroform (3×30 ml), dried over magnesium sulfate, and concentrated to give a crude product. Silica gel chromatography (ethyl acetate/hexanes, 4:6) afforded 0.75 g of the desired product as a faint yellow oil.

c)



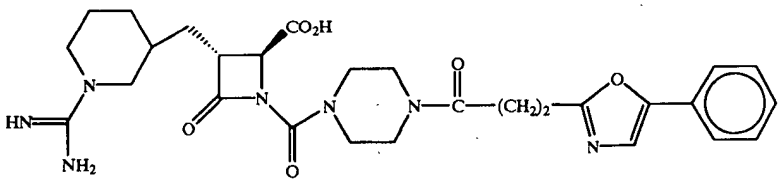
To a solution of the product from part (b) (0.70 g, 2.85 mmol) in dioxane/water (15.0/7.5 ml) was added 1N sodium hydroxide (7.1 ml, 7.1 mmol). The mixture was stirred until disappearance of the starting material. It was then concentrated, water (10 ml) was added, the mixture was cooled in an ice bath, and the pH was adjusted to 5.0 with the addition of 1N HCl to give a precipitate. The precipitate was collected and dried to give 0.26 g of the desired product as a white solid.

d)



To a solution of the product from part (c) (0.026 g, 0.12 mmol) and hydroxybenzotriazole (0.016 g, 0.016 mmol) in methylene chloride (3 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.023 g, 0.12 mmol) at 0° C. and stirred for 30 minutes. The HCl salt product from Example 203 (f) (0.075 g, 0.11 mmol) and diisopropylethylamine (0.034 g, 0.27 mmol) were added and the reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with methylene chloride and washed with 5% sodium bicarbonate, dried over magnesium sulfate, and concentrated to give a crude product. Silica gel chromatography (3% methanol/chloroform) afforded 0.053 g of the desired product as a colorless oil.

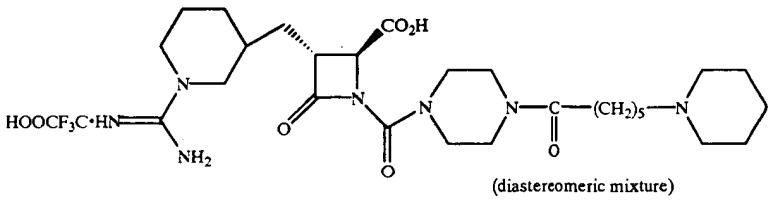
e)



A mixture of the product from part (d) (20 mg, 0.02 mmol), palladium on carbon catalyst (10%, 5 mg) in dioxane (1 ml) and 1N HCl (0.02 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for one hour. The reaction mixture was filtered through a Celite® pad and purified by preparative HPLC and lyophilized to give the desired product as a trifluroacetic acid salt.

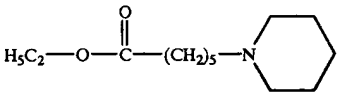
This salt was passed through a polyvinylpyridine column and lyophilized to afford 10 mg of the desired product (zwitterion) as a white lyophilate. IR (KBr) 1775 cm^{-1}; MS $(M+H)^+$=566.

EXAMPLE 205



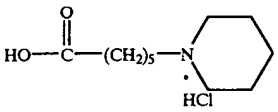
(diastereomeric mixture)

a)



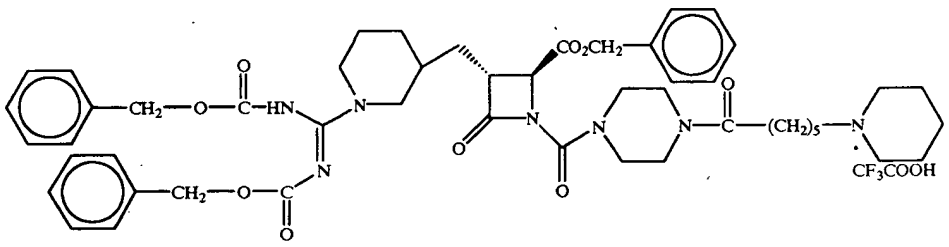
Ethyl 6-bromohexanote (8.00 ml, 44.82 mmol) was added to a solution of piperidine (11.98 ml, 121.01 mmol) in toluene (20 ml). The mixture was heated at 90° C., stirred for 2 hours, and then cooled to room temperature. The white precipitate was filtered out. The filtrate was diluted with ethyl acetate (60 ml), washed with water (2x30 ml) and brine (20 ml), and then acidified to pH 3.0 with 1N HCl. The aqueous layer was basified to pH 9.0 with solid potassium carbonate and extracted with ethyl acetate (3x30 ml). The combined ethyl acetate solution was washed with brine, dried over magnesium sulfate, and concentrated to give 10.0 g of the desired product as a colorless oil.

b)



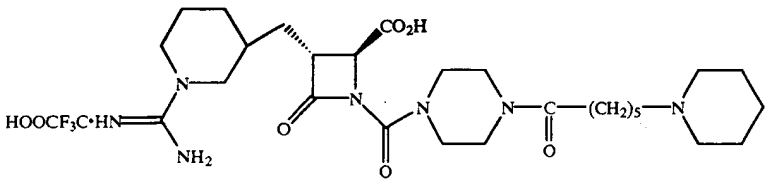
A solution of the product from part (a) (5.0 g, 0.022 mmol) in 5% sodium hydroxide ethanolic solution (100 ml) was stirred overnight and quenched with acetic acid (7.5 ml). The solvent was removed and the residue was dissolved in methylene chloride: methanol:acetic acid (2:1:1), flashed on a silica gel column (eluted with methylene chloride:methanol:acetic acid, 2:1:1) to give after concentration a colorless oil. This oil was dissolved in water (20 ml) and treated with concentrated HCl (25 ml). The extra HCl was removed under vacuum. The solution was lyophilized to provide 5.05 g of the desired product as a gray yellow solid.

c)



To a solution of the product from part (b) (25 mg, 0.11 mmol) and hydroxybenzotriazole (18 mg, 0.12 mmol) in methylene chloride (2 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (22 mg, 0.12 mmol) at 0° C. The mixture was stirred for 30 minutes and the HCl salt product from Example 203 (f) (72 mg, 0.11 mmol) and diisopropylethylamine (66 μl, 0.38 mmol) were added and the mixture was changed into a homogeneous solution. The solution was slowly warmed to room temperature within 3 hours, stirred overnight and concentrated. The residue was purified with preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid), and lyophilized to give 40 mg of the desired product as a white solid. MS $(M+H)^+$=906.

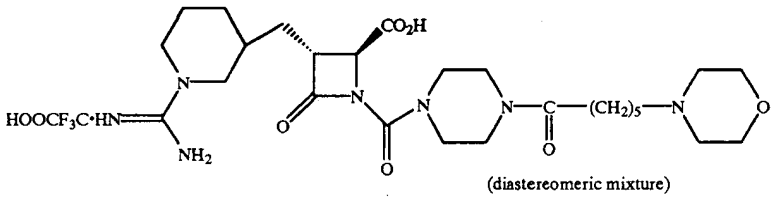
d)



A mixture of the product from part (c) (45 mg, 0.050 mmol), palladium on carbon catalyst (10%, 15 mg) and 1N HCl (100 μl, 0.10 mmol) in 1,4-dioxane (1.0 ml) was stirred under a hydrogen atmosphere (hydrogen balloon) at room temperature for 45 minutes. The reaction mixture was filtered through Celite® pad. The filtrate was concentrated.

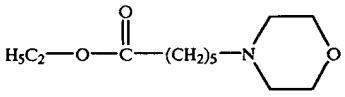
The residue was taken up with methanol:water (1:1), purified with preparative HPLC (reverse phase, methanol, water, trifluoroacetic acid), and lyophilized to give a white lyophilate. The lyophilate was dissolved in methanol: water (1:1) and passed through a polyvinylpyridine pad (eluted with methanol:water, 1:1). After methanol was removed, the solution was lyophilized to provide 8.3 mg of the desired product as a white powder (lyophilate). IR (KBr) 1776 cm^{-1}; MS $(M+H)^+$548.

EXAMPLE 206



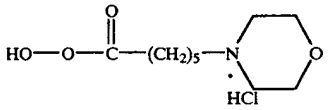
(diastereomeric mixture)

a)



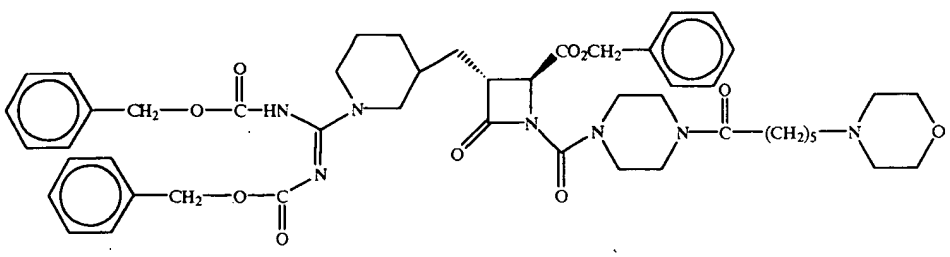
Following the procedure of Example 205(a) but employing morpholine in place of the piperidine, the desired product was obtained as a colorless oil.

b)



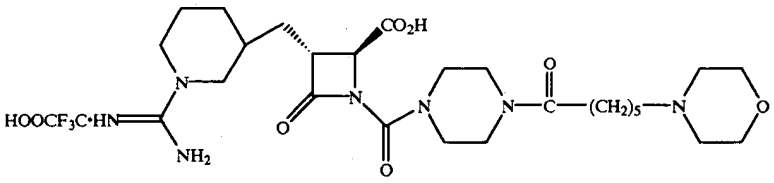
The product from part (a) was treated with a 5% sodium hydroxide ethanolic solution and concentrated HCl according to the procedure of Example 205(b) to give the desired product as a white solid following lyophilization.

c)



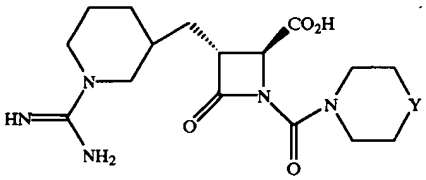
The product from part (b) was coupled to the HCl salt product from Example 203(f) according to the procedure of Example 205(c) to give the desired product as a white solid following lyophilization. MS(M+H)$^+$ 908.

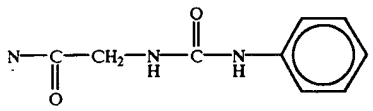
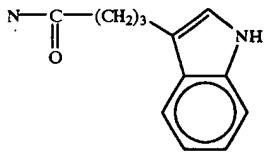
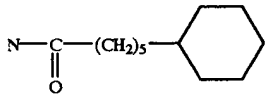
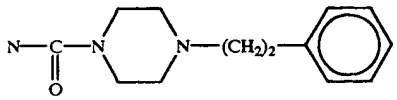
d)



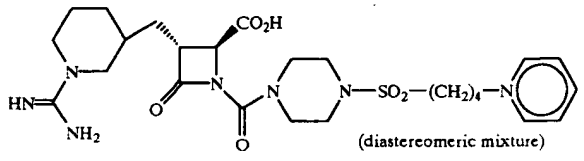
The product from part (c) was hydrogenated and purified according to the procedure of Example 205(d) to give the desired product as a white powder following lyophilization. IR(KBr) 1775 cm^{-1}; MS(M+H)$^+$=550.

The following procedure of Examples 204 to 206, the following compounds were also prepared:



Ex	Y	salt	stereochemistry	(M + H)$^+$
207		—	diastereomeric mixture	557
208		—	diastereomeric mixture	552
209		—	diastereomeric mixture	547
210		1.0 HCl	diastereomeric mixture	583

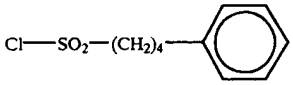
EXAMPLE 211



(diastereomeric mixture)

-continued

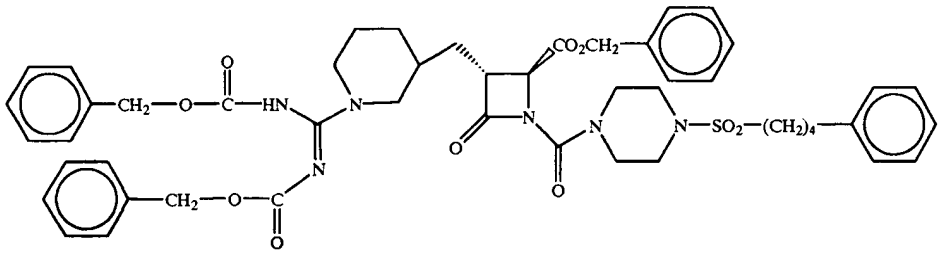
a)



A mixture of 4-phenyl-1-butanesulfonic acid mono sodium (65 mg, 0.275 mmol), thionyl chloride (0.81 mg,

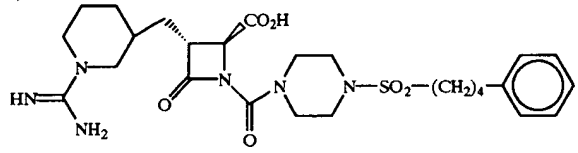
6.85 mmol), and dimethylformamide (a few drops) was heated at 65° C. for 3 hours. The mixture was cooled in an ice-bath and ice water was added to give a greenish residue. This residue was extracted with methylene chloride, dried over magnesium sulfate, and concentrated to give the desired product as a colorless oil.

b)



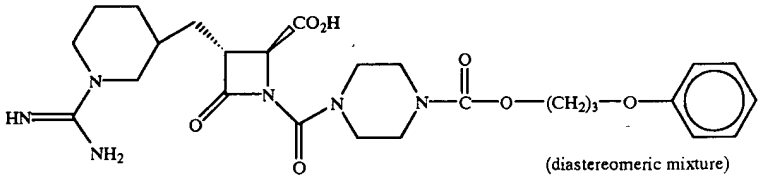
The product from part (a) (24.0 mg, 0.1 mmol) was added to a mixture of the product from Example 203 (d) (78.5 mg, 0.1 mmol) and potassium carbonate (41.5 mg, 0.3 mmol) in acetonitrile (1.0 ml) under argon at 0° C. and stirred at room temperature for 15 hours. The reaction mixture was then heated at 45° C. for 2 hours and triethylamine (30 mg, 0.3 mmol) and methylene chloride (0.5 ml) were added. The reaction mixture was then heated at 45° C. for 2 hours and concentrated in vacuo. Silica gel chromatography (methanol/chloroform, 1%) afforded 34 mg of the desired product as a clear oil.

c)



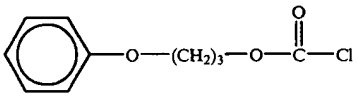
A mixture of the product from part (b) (34 mg, 0.037 mmol) and palladium on carbon catalyst (10%, 8 mg) in isopropanol (1 ml) and 1N HCl (0.11 ml) was stirred under hydrogen atmosphere (hydrogen balloon) at room temperature for 1 hour. Additional palladium on carbon catalyst (5 mg) was added and the mixture was stirred under hydrogen atmosphere for 1 hour. The reaction mixture was filtered through a Celite® pad, and purified by preparative HPLC and lyophilized to give the trifluoroacetic acid salt of the desired product. This salt was passed through a polyvinylpyridine column and lyophilized to afford 10.4 mg of the desired product (zwitterion) as a white lyophilate. IR(KBr) 1778 cm^{-1}; MS $(M+H)^+$=563.

EXAMPLE 212



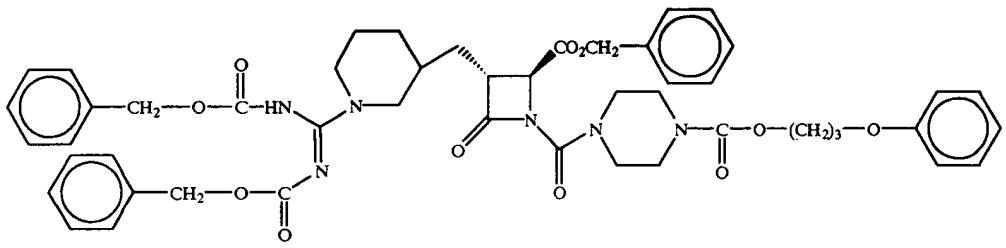
(diastereomeric mixture)

a)



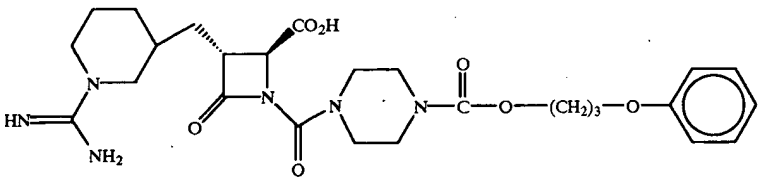
Sodium bicarbonate (1.0 g, 11.9 mmol) was added to a stirred solution of phenoxy-3-propanol (200 mg, 1.32 mmol) in methylene chloride (4 ml) at room temperature followed by phosgene (4.0 ml, 20% in toluene). An exothermic reaction occurred and the evolution of CO_2 gas was observed. After 30 minutes, the reaction mixture was filtered through a plug of silica gel to remove solids. The organic solution was concentrated to afford 265 mg of the desired product as a white solid.

b)



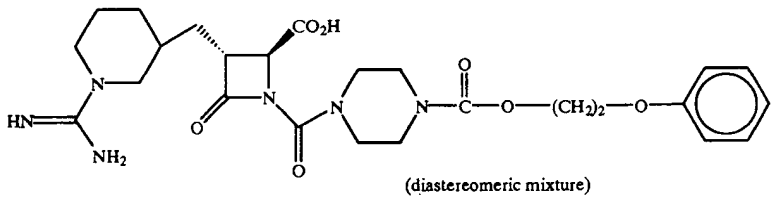
Triethylamine (35 μl, 0.25 mmol) and dimethylaminopyridine (1.5 mg, 0.01 mmol) were added to a stirred solution of the product from Example 203(d) (37 mg, 0.05 mmol) and the product from part (a) (15 mg, 0.07 mmol) in methylene chloride (3 ml) at room temperature. After 3 hours, the reaction mixture was directly purified by silica gel chromatography (50% ethyl acetate in hexanes) to give 42 mg of the desired product as a colorless oil.

c)



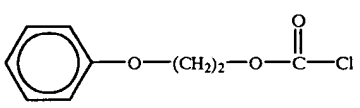
Palladium on carbon catalyst (10%, 20 mg) was added to a stirred solution of the product from part (b) (40 mg, 0.04 mmol) in a mixture of ethyl acetate (2 ml), ethanol (2 ml), and water (1 ml) at room temperature. Hydrogen gas was bubbled through the resulting suspension for 5 hours. After filtration through a plug of Celite® and concentration, the mixture was purified by preparative HPLC to afford the triluoroacetic acid salt of the desired product as a white solid. This trifluoroacetic acid salt was filtered through a column of polyvinylpyridine resin followed by lyophilization to give 18 mg of the desired product (zwitterion). IR(KBr) 1778 cm^{-1}; MS $(M+H)^+$=543.

EXAMPLE 213



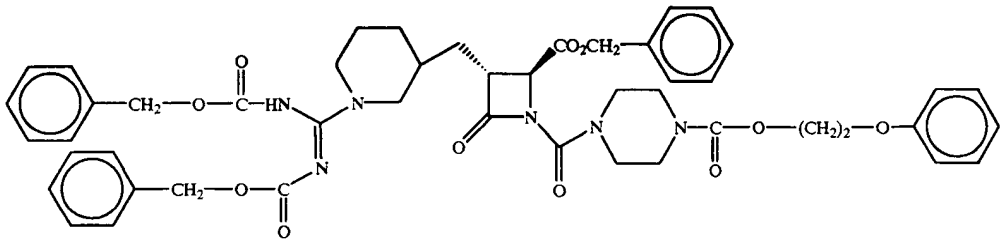
(diastereomeric mixture)

a)



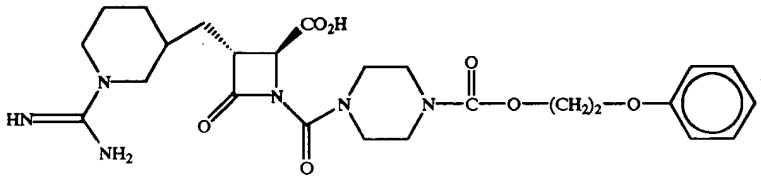
Following the procedure of Example 212(a) but employing phenoxy-2-ethanol, the desired compound was obtained as a white solid.

b)



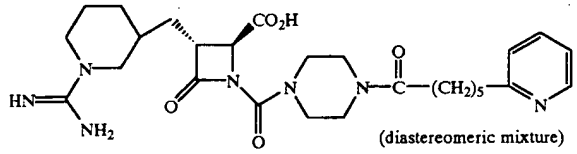
The product from part (a) was reacted with the product from Example 203(d) according the the procedure of Example 212(b) to give the desired product as a colorless oil.

c)



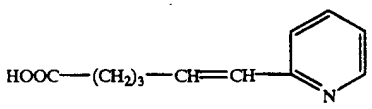
The product from part (b) was hydrogenated and worked-up according to the procedure of Example 212(c) to give the desired zwitterion product as a lyophilate. IR(KBr) 1776 cm^{-1}; MS $(M+H)^+$=543.

EXAMPLE 214



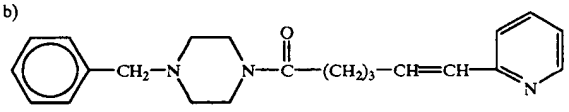
(diastereomeric mixture)

a)



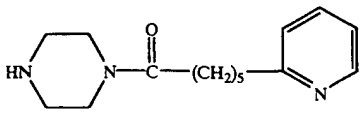
A suspsension of (4-carboxybutyl)triphenylphosphonium bromide (4.44 g, 10 mmol) in dry tetrahydrofuran (150 ml) was stirred at room temperature under an argon atmosphere and 2.5 equivalents of a 0.5 M solution of potassium bis(trimethylsilyl)amide (50 ml) in toluene was added. The resulting mixture was stirred overnight at room temperature and became a deep orange color. Pyridine-2-carboxaldehyde (1.18 g, 11 mmol) was added and the mixture was stirred for 3 hours. The reaction mixture was evaporated yielding a dark residue which was triturated with ether removing most of the triphenylphosphene oxide and giving 1.0 g of the desired product as a mixture of cis and trans isomers.

b)



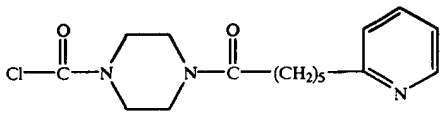
A mixture of the product from part (a) (900 mg), 1-benzylpiperazine (916 mg, 5.2 mmol), ethyl-3-(dimethylamino)propyl carbodiimide, hydrochloride salt (996 mg, 5.2 mmol), 1-hydroxybenzotriazole (700 mg, 5.2 mmol), and N-methylmorpholine (1.05 g, 10.4 mmol, 1.04 ml) in dimethylformamide (10 ml) was stirred at room temperature overnight under an argon atmosphere. The reaction was diluted with water and extracted with ethyl acetate, washed with water, 5% lithium chloride, and brine, and dried over anhydrous sodium sulfate. Evaporation yielded the crude product as a yellow oil. Purification by column chromatrography on silica, eluting with ethyl acetate/hexane (3:1) yielded 400 mg of the desired product.

c)



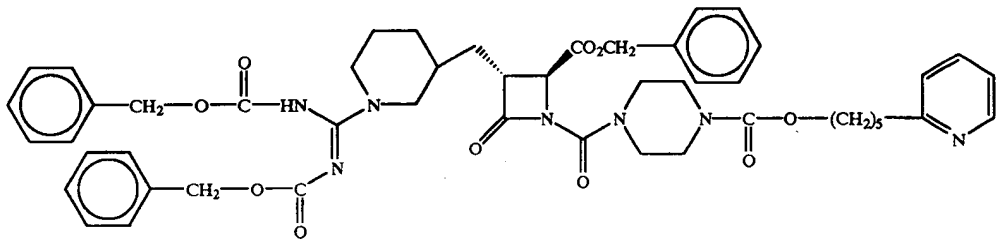
Platinum (IV) oxide (75 mg) was added to a solution of the product from part (b) (350 mg, 1.0 mmol) in ethanol (10 ml) and the resulting mixture was stirred under a hydrogen atomosphere overnight. The catalyst was removed by filtering through Celite® and the solvent was evaporated yielding 260 mg of the desired product as a colorless oil.

d)



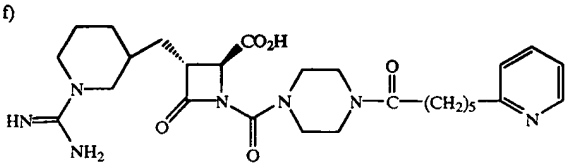
A solution of 3.5 equivalents of 20% phosgene in toluene (1.9 ml) and methylene chloride (15 ml) was cooled to 0° C. under an argon atmosphere and a solution of the product from part (c) (250 mg, 0.95 mmol) and triethylamine (135 mg, 1.33 mmol) in methylene chloride (15 ml) was added over 30 minutes. The reaction was stirred for 1 hour and was filtered, the solvents evaporated, and the crude product purified by column chromatography on silica eluting with 20% ethyl acetate/hexane yielding 150 mg of the desired product as a colorless solid.

e)



A mixture of the product from Example 203 (b) (245 mg, 0.4 mmol), the product from part (d) (130 mg, 0.4 mmol), diisopropylethylamine (51.7 mg, 0.4 mmol) and N,N-dimethylaminopyridine (5 mg) was stirred together in dry methylene chloride (1 ml). After stirring overnight at room temperature the mixture was evaporated and the crude product was purified by column chromatography eluting with 15% ethyl acetate/hexane yielding 142 mg of the desired product as a colorless glass-like residue. MS $(M+H)^+$=901.

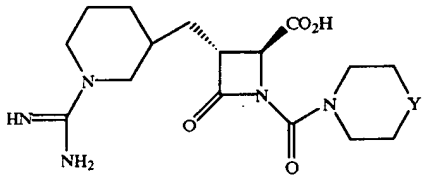
f)

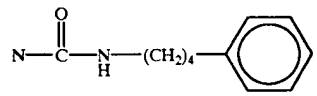
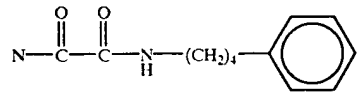


A solution of the product from part (e) (140 mg, 0.14 mmol) in dioxane (2 ml) containing 2.5 equivalents of 0.1N HCl and 10% palladium on carbon catalyst (20 mg) was stirred under a hydrogen atmosphere for 1.5 hours at room temperature. The reaction was filtered and passed through approximately 5 equivalents of polyvinylpyridine resin with water/dioxane (1:1). The solvents were removed by lyophilization yielding 56 mg of crude product as a colorless solid. Purification by preparative HPLC yielded 18 mg of colorless material, which was passed through a polyvinylpyridine resin yielding 9 mg of the desired product. MS $(M+H)^+$=542.

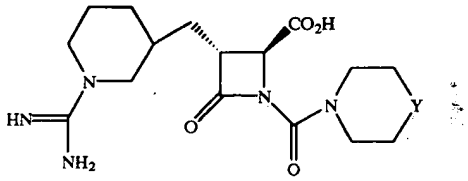
Examples 215–221

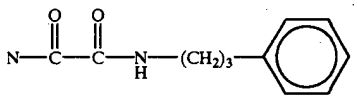
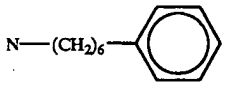
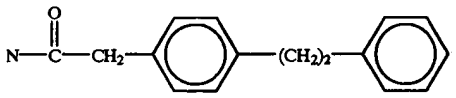
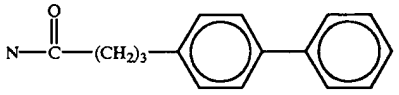
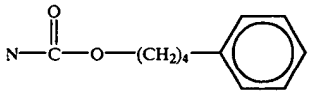
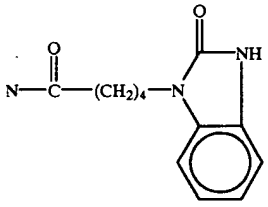
Following the procedure of Example 214, the following compounds were also prepared:



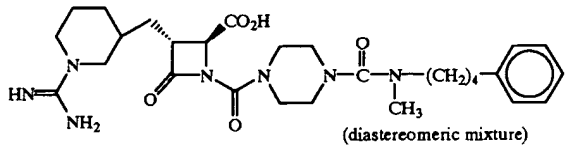
Ex	Y	salt	stereochemistry	$(M+H)^+$
215		—	diastereomeric mixture	542
216		—	diastereomeric mixture	570

-continued



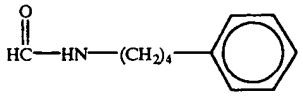
Ex	Y	salt	stereochemistry	(M + H)+
217		—	diastereomeric mixture	556
218		HCl	diastereomeric mixture	527
219		—	diastereomeric mixture	589
220		—	diastereomeric mixture	587
221		—	diastereomeric mixture	543
222		—	diastereomeric mixture	583

EXAMPLE 223



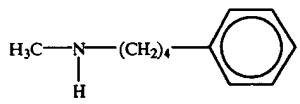
(diastereomeric mixture)

a)



Formic acid (1.50 ml, 42.88 mmol) was added dropwise to acetic anhydride (3.29 ml, 34.84 mmol) at 0° C., then the solution was gently heated (50–60° C., 2 hours). The mixture was cooled to room temperature. Tetrahydrofuran (5 ml) was added followed by the addition of a solution of 4-phenylbutylamine (2.0 g, 13.40 mmol) in tetrahydrofuran (10 ml). The solution was stirred for 3 hours. The volatiles were removed in vacuo to give 2.5 g of the crude desired product.

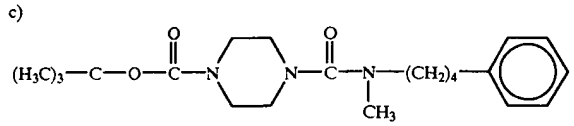
b)



The crude product from part (a) was dissolved in tetrahydrofuran (15 ml) and cooled to 0° C. Borane dimethylsulfide complex (3.35 ml, 33.50 mmol) was added dropwise. After vigorous reaction ceased, the resulting mixture was brought to a gentle reflux and maintained at that temperature until completion (about 3 hours). The reaction was cooled to 0° C. Methanol (10 ml) was added and the mixture was stirred for 1 hour. Anhydrous HCL was bubbled through the mixture to attain a pH less than or equal to 2.0. The mixture was gently refluxed for 1 hour and then cooled to room temperature. Methanol (20 ml) was added and the solvents were removed. The residue was made basic by adding aqueous sodium hydroxide and then extracted with ether (3x20 ml).

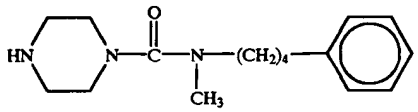
The combined ether layers were dried over magnesium sulfate and concentrated to give 2.2 of the desired product as a colorless oil. MS $(M+H)^+$=164.

c)



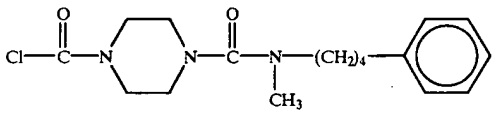
The carbamoyl chloride from Example 20(a) (19.43 mmol) was added to a solution of the product from part (b) (2.2 g, 13.50 mmol) and triethylamine (2.71 ml, 19.43) mmol) in methylene chloride (100 ml). The solution was stirred for 3 hours. The solvent was removed and the residue was purified by silica gel chromatography (hexane:ethyl acetate, 7:1 to 2:1) to give 3.90 g of the desired product as a colorless oil. MS $(M+H)^+$=376.

d)



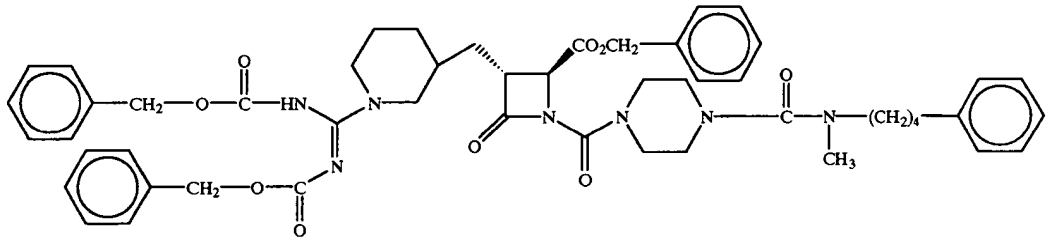
The product from part (c) was dissolved in methylene chloride (40 ml). The solution was cooled to 0° C. and trifluoroacetic acid (10 ml) was added dropwise. The ice-bath was removed. The mixture was stirred at room temperature for 1 hour. The solvents were removed under vacuum. The residue was diluted with ethyl acetate (50 ml). The solution was neutralized with saturated sodium bicarbonate (pH 10). The aqueous layer was extracted with ethyl acetate (2x30 ml). The combined ethyl acetate solution was washed with brine, dried over magnesium sulfate and concentrated to give the crude desired product as a colorless oil.

e)



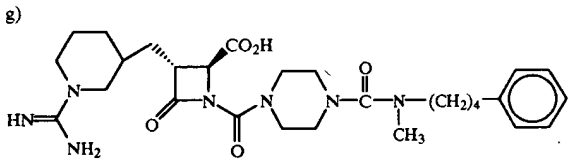
A solution of 20% phosgene in toluene (26.3 ml, 50 mmol) was dissolved in methylene chloride (15 ml) and cooled to 0° C. A solution of the crude product from part (d) and triethylamine in methylene chloride (15 ml) was added to the above solution over 10 minutes. The resulting solution was stirred at 0° C. for 1.5 hours. The precipitate was removed by filtration. The filtrate was concentrated. The residue was purified by silica gel chromatography (hexane:ethyl acetate, 3:1 to 2:1) to give 1.9 g of the desired product as a white solid.

f)



Triethylamine (0.018 ml, 0.13 mmol), the carbamoyl chloride product of part (e) (42.4 mg, 0.13 mmol) and 4-dimethylaminopyridine (1.0 mg, 0.007 mmol) were added to a solution of the product from Example 203(b) (60 mg, 0.10 mmol) in methylene chloride (2 ml). The solution was stirred for 3 hours. The solvent was removed and the residue was purified by silica gel chromatography (hexane:ethyl acetate, 4:3 to 1:1) to give 94 mg of the desired product as a colorless oil. MS $(M+H)^+$=914.

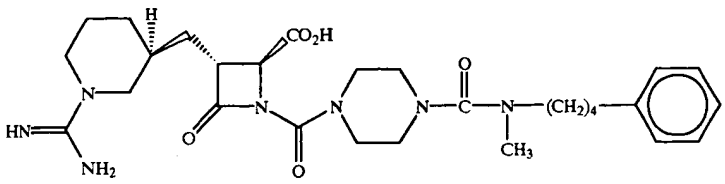
g)



A mixture of the product from part (f) (94 mg, 0.10 mmol), palladium on carbon catalyst (10%, 25 mg) and 1N HCl (100 μl, 0.10 mmol) in dioxane (2.0 ml) was stirred under a hydrogen atmosphere (hydrogen balloon) at room temperature for 45 minutes. HPLC indicated completion of the reaction. The reaction mixture was filtered through a Celite® pad and the filtrate was concentrated. The residue was taken up with methanol-water (1:1) and passed through a polyvinylpyridine pad (eluted with 1:1 methanol-water). After the methanol was removed, the solution was lyophilized to provide 46 mg of the desired product as a white lyophilate. IR(KBr) 1775 cm^{-1}; MS $(M+H)^+$=556.

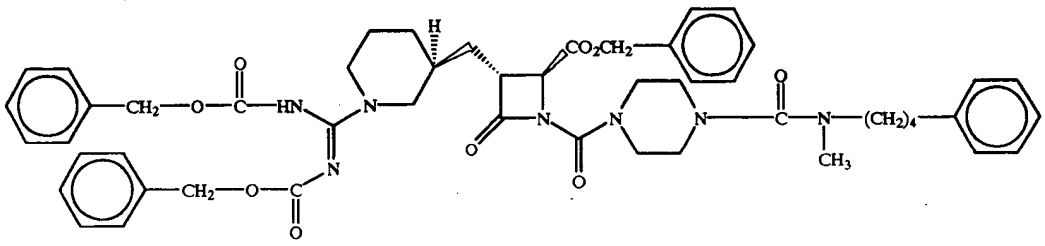
EXAMPLE 224

(2S,3R)-3-[[(3S)-1-(Aminoiminomethyl)-3-piperidinyl]methyl-1-[[4-[[methyl(4-phenylbutyl)amino]carbonyl-1-piperazinyl]carbonyl]-4-oxo-2-azetidinecarboxylic acid



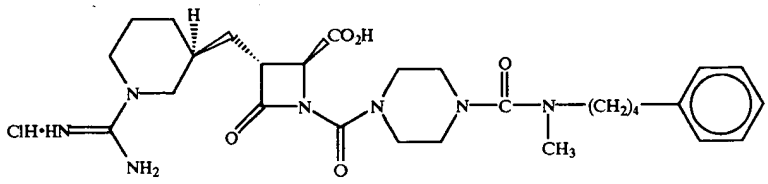
(homochiral)

a)



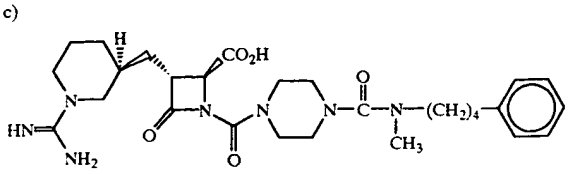
A solution of the product from Example 201(i) (613 mg, 1.0 mmol), triethylamine (0.30 ml, 2.15 mmol), the product of Example 223 (e) (422 mg, 1.25 mmol) and 4-dimethylaminopyridine (30.5 mg, 0.25 mmol) in methylene chloride (4 ml) was stirred under argon for 23 hours. The reaction was concentrated in vacuo and the residue was taken up in ethyl acetate, 10% potassium bisulfate, and water. The ethyl acetate layer was washed again with dilute potassium bisulfate, water (2×) and brine, dried over sodium sulfate, and concentrated to a foamy residue (1.03 g). Chromatography of the residue over silica gel using 60% and then 70% ethyl acetate in hexanes provided 803 mg of the desired product as an oily residue.

b)



The product from part (a) (777 mg, 0.85 mmol) was hydrogenated in dioxane (10 ml) and 1.0 N HCl (0.87 mmol) in the presence of 10% palladium on carbon catalyst (230 mg) at 1 atmosphere for 1.3 hours. After filtration using aqueous dioxane, the filtrate was concentrated to remove dioxane, filtered and lyophilized to give 492 mg of the desired product as a white solid.

c)

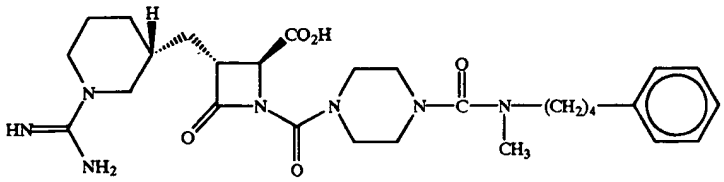


The product from part (b) (490 mg, 0.83 mmol) was passed through a column of 8 g of polyvinylpyridine resin packed in dioxane-water (30:70), and the product containing fractions were concentrated in vacuo and lyophilized to give 315 mg of the desired product as a white solid. IR(KBr) 1776 cm^{-1}; MS(M+H)$^+$=556.

Anal. calc'd for $C_{28}H_{41}N_7O_5 1.9\ H_2O 0.30$ dioxane: C, 56.90; H, 7.72; N, 15.91; H_2O, 5.55. Found: C, 56.49; H, 7.43; N, 15.92, H_2O, 4.77.

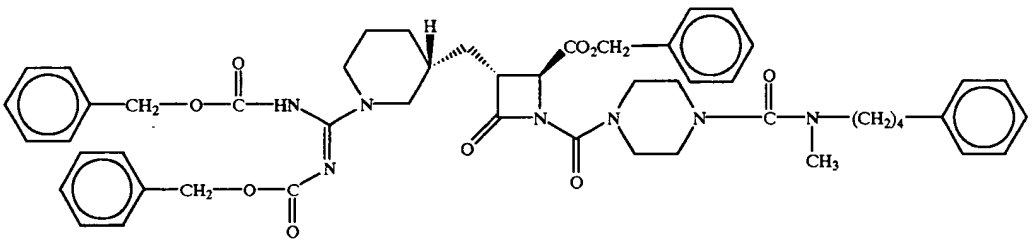
EXAMPLE 225

(2S,3R)-3-[[(3R)-1-(Aminoiminomethyl)-3-piperidinyl]methyl-1-[[4-[[methyl(4-phenylbutyl)amino]carbonyl-1-piperazinyl]carbonyl]-4-oxo-2-azetidinecarboxylic acid



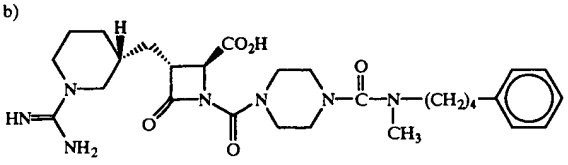
(homochiral)

a)



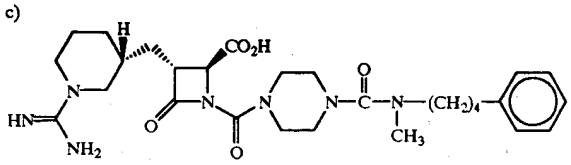
A solution of the product from Example 200 (i) (613 mg, 1.0 mmol) is Example 200(i) (613 mg, 1.0 mmol) is reacted with the product of Example 223(e) (422 mg, 1.25 mol) in the presence of triethylamine and 4-dimethylaminopyridine according to the procedure of Example 224 (a) to give 805 mg of the desired product as a foamy residue after chromatography over silica gel using 20% and then 30% ethyl acetate in methylene chloride.

b)



Hydrogenation of the product from part (a) according to the procedure of Example 224 (b) provided 460 mg of the desired product as a white solid after lyophilization.

c)



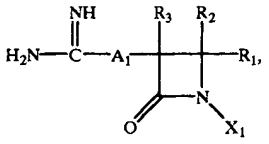
Passage of the product from part (b) (460 mg, 0.78 mmol) through a column of 8 g of polyvinylpyridine resin according to the procedure of Example 224(c) afforded after lyophilization 402 mg of the desired product as a white solid. IR(KBr) 1775 cm^{-1}; MS(M+H)$^+$=556.

Anal. calc'd for $C_{28}H_{41}N_7O_5.1.50\ H_2O 0.30$ dioxane: C, 57.58; H, 7.68; N, 16.10; H_2O, 4.44. Found: C, 57.24; H, 7.30; N, 16.37, H_2O, 3.99.

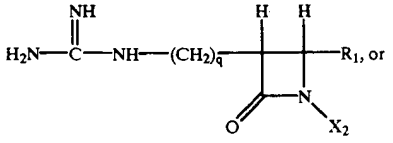
What is claimed is:

1. A compound of the formulas:

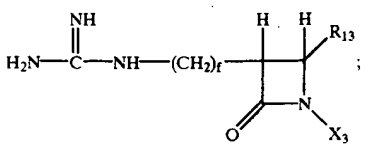
(I)



(IV)

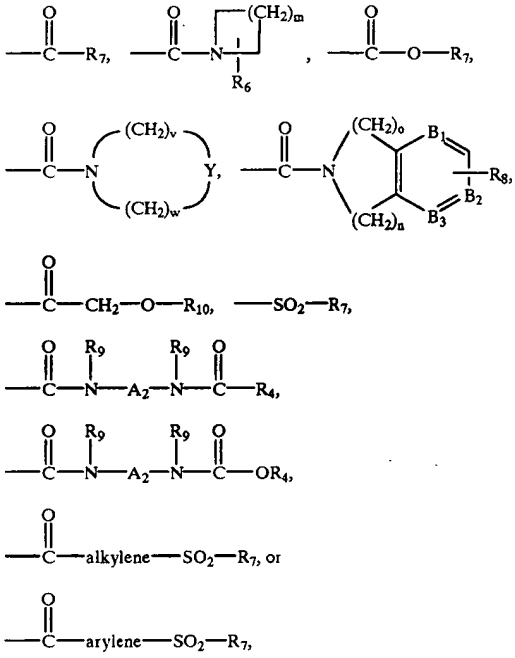


(V)



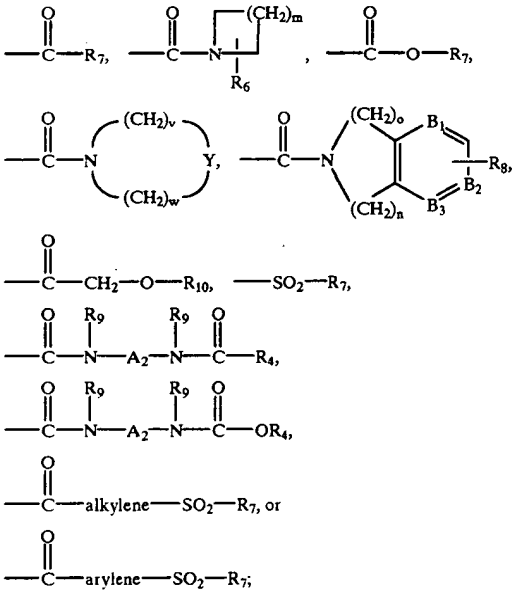
including an inner salt thereof, or a pharmaceutically acceptable salt thereof, or a hydrolyzable ester thereof, or a solvate thereof wherein:

R_1 is hydrogen, carboxy, alkoxycarbonyl, A_2-aryl,



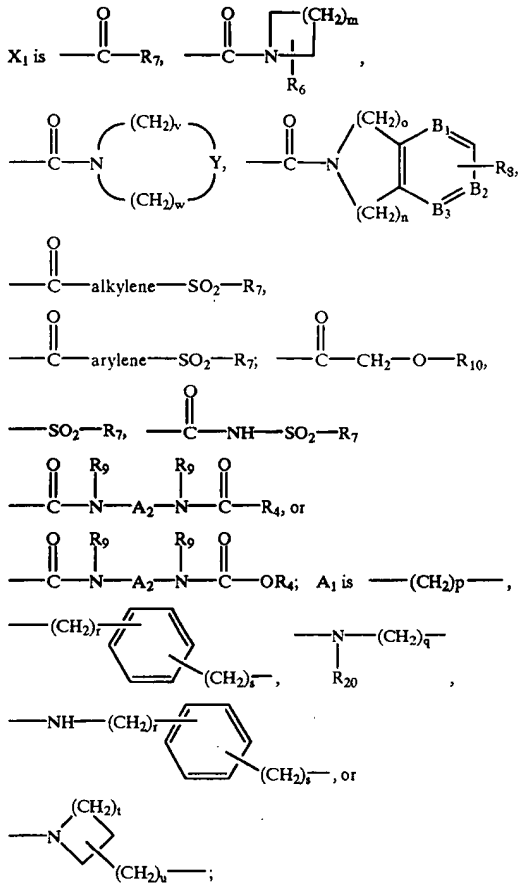
or R_1 is alkyl provided that R_2 is alkyl and R_3 is hydrogen;

R_1' is carboxy, alkoxycarbonyl, A_2-aryl,



R_2 and R_3 are both hydrogen, or R_2 is alkyl provided that R_3 is hydrogen, or R_3 is alkyl provided that R_2 is hydrogen;

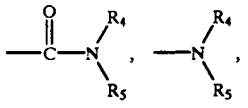
X_1 is



R_4 and R_5 are independently selected from hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl A_2-cycloalkyl, A_2-substituted cycloalkyl, aryl, substituted aryl, A_2-aryl, A_2-substituted aryl, heteroaryl, A_2-heteroaryl, heterocycloalkyl, A_2-heterocycloalkyl, arylene-A_3-aryl, A_2-arylene-A_3-aryl, arylene-A_3-cycloalkyl, A_2-arylene-A_3-cycloalkyl, arylene-A_3-heteroaryl, A_2-arylene-A_3-heteroaryl, arylene-A_3-heterocycloalkyl, A_2-arylene-A_3-heterocycloalkyl, arylene-A_3-substituted aryl, A_2-arylene-A_3-substitued aryl, arylene-A_3-substituted cycloalkyl, A_2-arylene-A_3-substituted cycloalkyl, cycloalkylene-A_3-cycloalkyl, A_2-cycloalkylene-A_3-cycloalkyl, cycloalkylene-A_3-aryl, A_2-cycloalkylene-A_3-aryl, cycloalkylene-A_3-heteroaryl, A_2-cycloalkylene-A_3-heteroaryl, cycloalkylene-A_3-heterocycloalkyl, A_2-cycloalkylene-A_3-heterocycloalkyl, cycloalkylene-A_3-substituted cycloalkyl, A_2-cycloalkylene-A_3-substituted cycloalkyl, cycloalkylene-A_3-substituted aryl, A_2-cycloalkylene-A_3-substituted aryl, substituted cycloalkylene-A_3-cycloalkyl, A_2-substituted cycloalkylene-A_3-cycloalkyl, substituted cycloalkylene-A_3-substituted cycloalkyl, A_2-substituted cycloalkylene-A_3-substituted cycloalkyl, substituted cycloalkylene-A_3-aryl, A_2-substituted cycloalkylene-A_3-aryl, substituted cycloalkylene-A_3-heteroaryl, A_2-substituted cycloalkylene-A_3-heteroaryl, substituted cycloalkylene-A_3-heterocycloalkyl, A_2-substituted cycloalkylene-A_3-heterocycloalkyl, substituted

cycloalkylene-A_3-substituted aryl, A_2-substituted cycloalkylene-A_3-substituted aryl, heteroarylene-A_3-heteroaryl, A_2-heteroarylene-A_3-heteroaryl, heteroarylene-A_3-cycloalkyl, A_2-heteroarylene-A_3-cycloalkyl, heteroarylene-A_3-substituted cycloalkyl, A_2-heteroarylene-A_3-substituted cycloalkyl, heteroarylene-A_3-aryl, A_2-heteroarylene-A_3-aryl, heteroarylene-A_3-heterocycloalkyl, A_2-heteroarylene-A_3-heterocycloalkyl, heteroarylene-A_3-substituted aryl, A_2-heteroarylene-A_3-substituted aryl, heterocycloalkylene-A_3-heterocycloalkyl, A_2-heterocycloalkylene-A_3-heterocycloalkyl, heterocycloalkylene-A_3-cycloalkyl, A_2-heterocycloalkylene-A_3-cycloalkyl, heterocycloalkylene-A_3-substituted cycloalkyl, A_2-heterocycloalkylene-A_3-substituted cycloalkyl, heterocycloalkylene-A_3-aryl, A_2-heterocycloalkylene-A_3-aryl, heterocycloalkylene-A_3-substituted aryl, A_2-heterocycloalkylene-A_3-substituted aryl, heterocycloalkylene-A_3-heteroaryl, A_2-heterocycloalkylene-A_3-heteroaryl, substituted arylene-A_3-substituted aryl, A_2-substituted arylene-A_3-substituted aryl, substituted arylene-A_3-cycloalkyl, A_2-substituted arylene-A_3-cycloalkyl, substituted arylene-A_3-substituted cycloalkyl, A_2-substituted arylene-A_3-substituted cycloalkyl, substituted arylene-A_3-aryl, A_2-substituted arylene-A_3-aryl, substituted arylene-A_3-heteroaryl, A_2-substituted arylene-A_3-heteroaryl, substituted arylene-A_3-heterocycloalkyl, and A_2-substituted arylene-A_3-heterocycloalkyl;

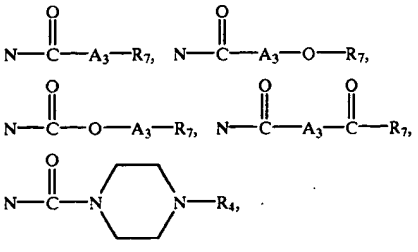
R_6 is hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, A_2-cycloalkyl, A_2-substituted cycloalkyl, aryl, substituted aryl, A_2-aryl, A_2-substituted aryl, arylene-A_3-aryl, A_2-arylene-A_3-aryl, heteroaryl, A_2-heteroaryl, heterocycloalkyl, A_2-heterocycloalkyl, arylene-A_3-cycloalkyl, A_2-arylene-A_3-cycloalkyl, arylene-A_3-heteroaryl, A_2-arylene-A_3-heteroaryl, arylene-A_3-heterocycloalkyl, A_2-arylene-A_3-heterocycloalkyl, carboxy, alkoxycarbonyl, arloxycarbonyl,



alkoxycarbonylamino, aryloxycarbonylamino, arylcarbonylamino, —N(alkyl)(alkoxycarbonyl), —N(alkyl)(aryloxycarbonyl), alkylcarbonylamino, —N(alkyl)(alkylcarbonyl), or —N(alkyl)(arylcarbonyl);

m is an integer from 1 to 5;

Y is O, S, N—R_4, N—SO_2—R_7,



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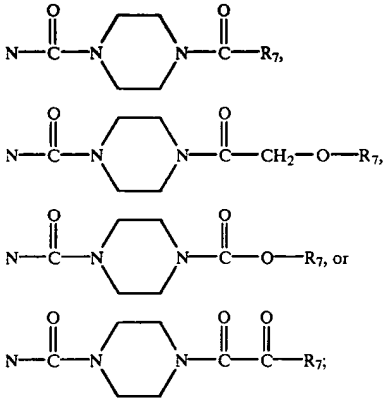
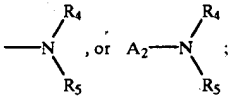


R_7 is alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, A_2-cycloalkyl, A_2-substituted cycloalkyl, aryl, substituted aryl, A_2-aryl, A_2-substituted aryl, heteroaryl, A_2-heteroaryl, heterocycloalkyl, A_2-heterocycloalkyl, arylene-A_3-aryl, A_2-arylene-A_3-aryl, arylene-A_3-cycloalkyl, A_2-arylene-A_3-cycloalkyl, arylene-A_3-heteroaryl, A_2-arylene-A_3-heteroaryl, arylene-A_3-heterocycloalkyl, A_2-arylene-A_3-heterocycloalkyl, arylene-A_3-substituted aryl, A_2-arylene-A_3-substitued aryl, arylene-A_3-substituted cycloalkyl, A_2-arylene-A_3-substituted cycloalkyl, cycloalkylene-A_3-cycloalkyl, A_2-cycloalkylene-A_3-cycloalkyl, cycloalkylene-A_3-aryl, A_2-cycloalkylene-A_3-aryl, cycloalkylene-A_3-heteroaryl, A_2-cycloalkylene-A_3-heteroaryl, cycloalkylene-A_3-heterocycloalkyl, A_2-cycloalkylene-A_3-heterocycloalkyl, cycloalkylene-A_3-substituted cycloalkyl, A_2-cycloalkylene-A_3-substituted cycloalkyl, cycloalkylene-A_3-substituted aryl, A_2-cycloalkylene-A_3-substituted aryl, substituted cycloalkylene-A_3-cycloalkyl, A_2-substituted cycloalkylene-A_3-cycloalkyl, substituted cycloalkylene-A_3-substituted cycloalkyl, A_2-substituted cycloalkylene-A_3-substituted cycloalkyl, substituted cycloalkylene-A_3-aryl, A_2-substituted cycloalkylene-A_3-aryl, substituted cycloalkylene-A_3-heteroaryl, A_2-substituted cycloalkylene-A_3-heteroaryl, substituted cycloalkylene-A_3-heterocycloalkyl, A_2-substituted cycloalkylene-A_3-heterocycloalkyl, substituted cycloalkylene-A_3-substituted aryl, A_2-substituted cycloalkylene-A_3-substituted aryl, heteroarylene-A_3-heteroaryl, A_2-heteroarylene-A_3-heteroaryl, heteroarylene-A_3-cycloalkyl, A_2-heteroarylene-A_3-cycloalkyl, heteroarylene-A_3-substituted cycloalkyl, A_2-heteroarylene-A_3-substituted cycloalkyl, heteroarylene-A_3-aryl, A_2-heteroarylene-A_3-aryl, heteroarylene-A_3-heterocycloalkyl, A_2-heteroarylene-A_3-heterocycloalkyl, heteroarylene-A_3-substituted aryl, A_2-heteroarylene-A_3-substituted aryl, heterocycloalkylene-A_3-heterocycloalkyl, A_2-heterocycloalkylene-A_3-heterocycloalkyl, heterocycloalkylene-A_3-cycloalkyl, A_2-heterocycloalkylene-A_3-cycloalkyl, heterocycloalkylene-A_3-substituted cycloalkyl, A_2-heterocycloalkylene-A_3-substituted cycloalkyl, heterocycloalkylene-A_3-aryl, A_2-heterocycloalkylene-A_3-aryl, heterocycloalkylene-A_3-substituted aryl, A_2-heterocycloalkylene-A_3-substituted aryl, heterocycloalkylene-A_3-heteroaryl, A_2-

heterocycloalkylene-A_3-heteroaryl, substituted arylene-A_3-substituted aryl, A_2-substituted arylene-A_3-substituted aryl, substituted arylene-A_3-cycloalkyl, A_2-substituted arylene-A_3-cycloalkyl, substituted arylene-A_3-substituted cycloalkyl, A_2-substituted arylene-A_3-substituted cycloalkyl, substituted arylene-A_3-aryl, A_2-substituted arylene-A_3-aryl, substituted arylene-A_3-heteroaryl, A_2-substituted arylene-A_3-heteroaryl, substituted arylene-A_3-heterocycloalkyl, A_2-substituted arylene-A_3-heterocycloalkyl,



n and o are one or two provided that the sum of n plus o is two or three;

v and w are one, two, or three provided that the sum of v plus w is three, four, or five;

R_8 is hydrogen, halo, amino, —NH(lower alkyl), —N(lower alkyl)$_2$, nitro, alkyl, substituted alkyl, alkoxy, hydroxy, aryl, substituted aryl, A_2-aryl, A_2-substituted aryl, arylene-A_3-aryl, A_2-arylene-A_3-aryl, cycloalkyl, substituted cycloalkyl, A_2-cycloalkyl, A_2-substituted cycloalkyl, heteroaryl, A_2-heteroaryl, heterocycloalkyl, A_2-heterocycloalkyl, arylene-A_3-cycloalkyl, A_2-arylene-A_3-cycloalkyl, arylene-A_3-heteroaryl, A_2-arylene-A_3-heteroaryl, arylene-A_3-heterocycloalkyl, or A_2-arylene-A_3-heterocycloalkyl;

B_1, B_2 and B_3 are each CH, or two of B_1, B_2 and B_3 are CH and the other is N, or one of B_1, B_2 and B_3 is CH and the other two are N;

R_9 is hydrogen or lower alkyl;

R_{10} is alkyl, substituted alkyl, alkylene-O-alkyl, alkylene-O-alkylene-O-alkyl, cycloalkyl, substituted cycloalkyl, A_2-cycloalkyl, A_2-substituted cycloalkyl, aryl, substituted aryl, A_2-aryl, A_2-substituted aryl, arylene-A_3-aryl, A_2-arylene-A_3-aryl, heteroaryl, A_2-heteroaryl, heterocycloalkyl, A_2-heterocycloalkyl, arylene-A_3-cycloalkyl, A_2-arylene-A_3-cycloalkyl, arylene-A_3-heteroaryl, A_2-arylene-A_3-heteroaryl, arylene-A_3-heterocycloalkyl or A_2-arylene-A_3-heterocycloalkyl;

R_{20} is alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, A_2-cycloalkyl, A_2-substituted cycloalkyl, A_2-aryl, or A_2-substituted aryl;

R_{21}, and R_{22} are independently selected from hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, A_2-cycloalkyl, A_2-substituted cycloalkyl, A_2-aryl, and A_2-substituted aryl;

p is an integer from 2 to 6;

q is an integer from 1 to 6;

f is an integer from 3 to 5;

r is zero, one or two;

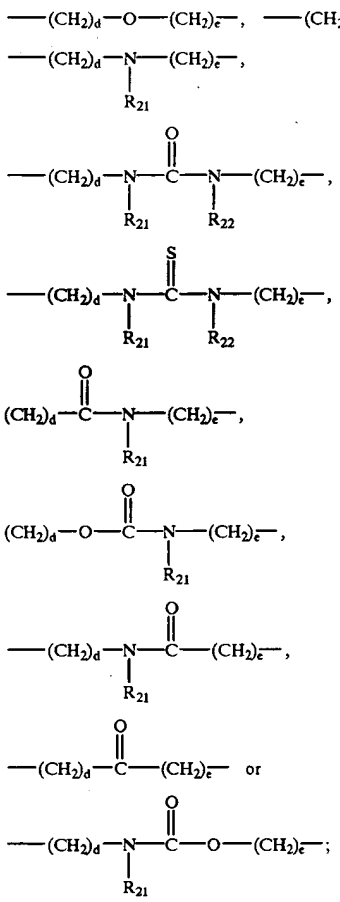
s is one or two;

t is one, two, three or four;

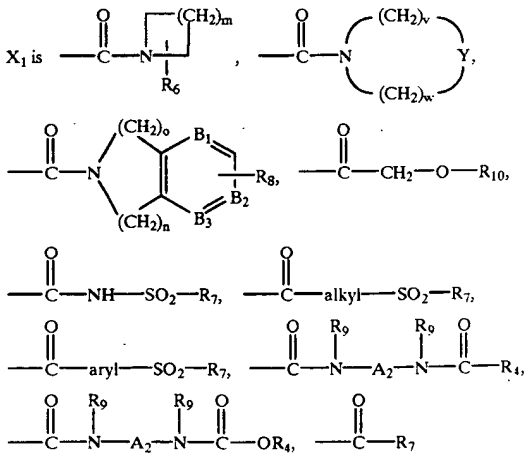
u is one, two or three;

A_2 is an alkylene or a substituted alkylene bridge of 1 to 10 carbons, an alkenylene or substituted alkenylene bridge of 2 to 10 carbons having one or more double bonds, or an alkynylene or substituted alkynylene bridge of 2 to 10 carbons having one or more triple bonds;

A_3 is a bond, an alkylene or a substituted alkylene bridge of 1 to 10 carbons, an alkenylene or substituted alkenylene bridge of 2 to 10 carbons having one or more double bonds, an alkynylene or substituted alkynylene bridge of 2 to 10 carbons having one or more triple bonds,



d and e are independently selected from zero and an integer from 1 to 10 provided that the sum of d plus e is no greater then 10;



US006376472B1

(12) **United States Patent**
Myers et al.

(10) **Patent No.: US 6,376,472 B1**
(45) **Date of Patent: Apr. 23, 2002**

(54) **COMPOUNDS HAVING ANTIHYPERTENSIVE, CARDIOPROTECTIVE, ANTI-ISCHEMIC AND ANTILIPOLYTIC PROPERTIES**

(75) Inventors: **Michael R. Myers**, Reading, PA (US); **Martin P. Maguire**, Woburn, MA (US); **Alfred P. Spada**, Lansdale, PA (US); **William R. Ewing**, Downingtown, PA (US); **Henry W. Pauls**; **Yong Mi Choi-Sledeski**, both of Collegeville, PA (US)

(73) Assignee: **Aventis Pharmaceuticals, Inc.**, Bridgewater, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/174,191**

(22) Filed: **Oct. 16, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US97/11320, filed on Jul. 1, 1997
(60) Provisional application No. 60/021,366, filed on Jul. 8, 1996.

(51) **Int. Cl.**[7] **C07D 473/34**; C07D 471/04; A61K 31/52; A61K 31/437; A61P 9/10

(52) **U.S. Cl.** **514/44**; 536/27.14; 536/27.3; 536/27.62; 544/277

(58) **Field of Search** 544/277; 536/27.14, 536/27.3, 27.62; 514/46, 266

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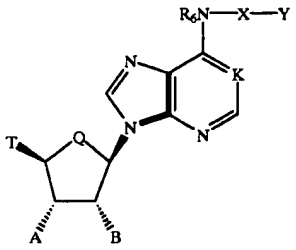
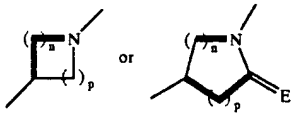
Primary Examiner—Mark L. Berch
(74) *Attorney, Agent, or Firm*—Paul R. Darkes; Peter Butch; Irving Newman

(57) **ABSTRACT**

A compound of the formula



wherein K is N; Q is CH_2 or O; R_6 is hydrogen, alkyl, allyl, 2-methylallyl, 2-butenyl, or cycloalkyl

X is  or

where the nitrogen of the ring of X is substituted by Y; E is O or S; Y is hydrogen, alkyl, aralkyl, substituted aralkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, or substituted heterocyclylalkyl; and n and p are independently 0, 1, 2, or 3, provided that n+p is at least 1;

T is hydrogen, alkyl, alkylcarbonyl, alkylthiocarbonyl, halo, carboxyl,

A and B are independently hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, or OR';

or a pharmaceutically acceptable salt thereof, a pharmaceutic-ally acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

15 Claims, No Drawings

COMPOUNDS HAVING ANTIHYPERTENSIVE, CARDIOPROTECTIVE, ANTI-ISCHEMIC AND ANTILIPOLYTIC PROPERTIES

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of International Patent Application Number PCT/US97/11320, filed Jul. 1, 1997, which claims the benefit of U.S. Ser. No. 60/021,366, filed Jul. 8, 1996, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to compounds derived from adenosine and analogues thereof, to pharmaceutical compositions containing such compounds, to their use in treating hypertension and myocardial ischemia, to their use as cardioprotective agents which ameliorate ischemic injury or myocardial infarct size consequent to myocardial ischemia, and to their use as antilipolytic agents which reduce plasma lipid levels, serum triglyceride levels, and plasma cholesterol levels, and to methods and intermediates used in the preparation of such compounds.

Hypertension

Hypertension, a condition of elevated blood pressure, affects a substantial number of the human population. Consequences of persistent hypertension include vascular damage to the ocular, renal, cardiac and cerebral systems, and the risk of these complications increases as blood pressure increases. Basic factors controlling blood pressure are cardiac output and peripheral vascular resistance, with the latter being the predominant common mechanism which is controlled by various influences. The sympathetic nervous system regulates peripheral vascular resistance through direct effects on alpha- and beta-adrenergic receptors as well as through indirect effects on renin release. Drug therapy is aimed at specific components of these blood pressure regulatory systems, with different mechanisms of action defining the several drug classes including diuretics, beta-adrenergic receptor antagonists (beta-blockers), angiotensin-converting enzyme (ACE) inhibitors, and calcium channel antagonists.

Thiazide-type diuretics are used in hypertension to reduce peripheral vascular resistance through their effects on sodium and water excretion. This class of drugs includes hydrochlorothiazide, chlorothiazide, methyclothiazide, and cyclothiazide, as well as related agents indapamide, metolazone, and chlorthalidone. Although the beta-blocker mechanism of action was once believed to be blockade of the $beta_1$-adrenergic receptor subtype in the heart to reduce heart rate and cardiac output, more recent beta-blockers with intrinsic sympathomimetic activity (ISA), including pindolol, acebutolol, penbutolol, and carteolol, are as effective as non-ISA beta-blockers, causing less reduction in heart rate and cardiac output. Other postulated mechanisms for these drugs include inhibition of renin release, a central effect, and an effect at pre-synaptic beta-adrenergic receptors resulting in inhibition of norepinephrine release. Cardioselective beta-blockers metoprolol (Lopressor-Geigy), acebutolol (Sectral-Wyeth), and atenolol (Tenormin-1CI), at low doses, have a greater effect on $beta_1$-adrenergic receptors than on $beta_2$-adrenergic receptor subtypes located in the bronchi and blood vessels. Nonselective beta-blockers act on both beta-adrenergic receptor subtypes and include propranolol (Inderal-Ayerst), timolol (Blocadren-Merck), nadolol (Corgard-Squibb), pindolol (Visken-Sandoz), penbutolol (Levatol-Hoechst-Roussel), and carteolol (Cartrol-Abbott). Adverse effects of beta-blockers include asymptomatic bradycardia, exacerbation of congestive heart failure, gastrointestinal disturbances, increased airway resistance, masked symptoms of hypoglycemia, and depression. They may cause elevation of serum triglycerides and may lower high-density lipoprotein cholesterol.

ACE inhibitors prevent the formation of angiotensin II and inhibit breakdown of bradykinin. Angiotensin II is a potent vasoconstrictor and also stimulates the secretion of aldosterone. By producing blockade of the renin-angiotensin-aldosterone system, these agents decrease peripheral vascular resistance, as well as sodium and water retention. In addition, ACE inhibitors increase levels of bradykinin and prostaglandins, endogenous vasodilators. Captopril (Capoten-Squibb) and Enalapril (Vasotec-Merck) are the leading ACE inhibitors. Adverse effects of the ACE inhibitors include rash, taste disturbance, proteinuria, and neutropenia.

The calcium channel antagonists reduce the influx of calcium into vascular smooth muscle cells and produce systemic vasodilation, resulting in their antihypertensive effect. Other effects of calcium channel antagonists include interference with action of angiotensin II and $alpha_2$-adrenergic receptor blockade, which may add to their antihypertensive effects. Calcium channel antagonists do not have the adverse metabolic and pharmacologic effects of thiazides or beta-blockers and may therefore be useful in patients with diabetes, peripheral vascular disease, or chronic obstructive pulmonary disease. Two calcium channel antagonists, Verapamil and diltiazem, have serious adverse cardiovascular effects on atrioventricular cardiac conduction in patients with preexisting conduction abnormalities, and they may worsen bradycardia, heart block, and congestive heart failure. Other minor adverse effects of calcium channel antagonists include peripheral edema, dizziness, light-headedness, headache, nausea, and flushing, especially with nifedipine and nicardipine.

Many other agents are available to treat essential hypertension. These agents include prazosin and terazocin, $alpha_1$-adrenergic receptor antagonists whose antihypertensive effects are due to resultant arterial vasodilation; clonidine, an $alpha_2$-adrenergic agonist which acts centrally as well as peripherally at inhibitory $alpha_2$-adrenergic receptors, decreasing sympathetic response. Other centrally acting agents include methyldopa, guanabenz, and guanfacine; reserpine, which acts by depleting stores of catecholamines; guanadrel, a peripheral adrenergic antagonist similar to guanethidine with a shorter duration of action; and direct-acting vasodilators such as hydralazine and minoxidil. These agents, although effective, produce noticeable symptomatic side effects, including reflex sympathetic stimulation and fluid retention, orthostatic hypotension, and impotence.

Many antihypertensive agents activate compensatory pressor mechanisms, such as increased renin release, elevated aldosterone secretion and increased sympathetic vasoconstrictor tone, which are designed to return arterial pressure to pretreatment levels, and which can lead to salt and water retention, edema and ultimately to tolerance to the antihypertensive actions of the agent. Furthermore, due to the wide variety of side effects experienced with the present complement of antihypertensive drugs and the problems experienced therewith by special populations of hypertensive patients, including the elderly, blacks, and patients with

chronic obstructive pulmonary disease, diabetes, or peripheral vascular diseases, there is a need for additional classes of drugs to treat hypertension.

Ischemia

Myocardial ischemia is the result of an imbalance of myocardial oxygen supply and demand and includes exertional and vasospastic myocardial dysfunction. Exertional ischemia is generally ascribed to the presence of critical atherosclerotic stenosis involving large coronary arteries resulting in a reduction in subendocardial flow. Vasospastic ischemia is associated with a spasm of focal variety, whose onset is not associated with exertion or stress. The spasm is better defined as an abrupt increase in vascular tone. Mechanisms for vasospastic ischemia include: (i) Increased vascular tone at the site of stenosis due to increased catecholamine release: (ii) Transient intraluminal plugging and (iii) Release of vasoactive substances formed by platelets at the site of endothelial lesions.

The coronary circulation is unique since it perfuses the organ which generates the perfusion pressure for the entire circulation. Thus, interventions which alter the state of the peripheral circulation and contractility will have a profound effect on coronary circulation. The regulatory component of the coronary vasculature is the small coronary arterioles which can greatly alter their internal diameter. The alteration of the internal radius is the result of either intrinsic contraction of vascular smooth muscle (autoregulation) or extravascular compression due to ventricular contraction. The net effect of therapies on the ischemic problem involves a complex interaction of opposing factors which determine the oxygen supply and demand.

Cardioprotection and Prevention of Ischemic Injury

The development of new therapeutic agents capable of limiting the extent of myocardial injury, i.e., the extent of myocardial infarction, following acute myocardial ischemia is a major concern of modern cardiology.

The advent of thrombolytic (clot dissolving) therapy during the last decade demonstrates that early intervention during heart attack can result in significant reduction of damage to myocardial tissue. Large clinical trials have since documented that thrombolytic therapy decreases the risk of developing disturbances in the heartbeat and also maintains the ability of the heart to function as a pump. This preservation of normal heart function has been shown to reduce long-term mortality following infarction.

There has also been interest in the development of therapies capable of providing additional myocardial protection which could be administered in conjunction with thrombolytic therapy, or alone, since retrospective epidemiological studies have shown that mortality during the first few years following infarction appears to be related to original infarct size.

In preclinical studies of infarction, conducted in a variety of animal models, many types of pharmacological agents such as calcium channel blockers, prostacyclin analogues, and agents capable of inhibiting certain metabolic pathways have been shown to be capable of reducing ischemic injury in several animal species.

Recent studies have demonstrated that exposure of the myocardium to brief periods of ischemia (interruption of blood flow to the heart) followed by reperfusion (restoration of blood flow) is able to protect the heart from the subsequent ischemic injury that would otherwise result from subsequent exposure to a longer period of ischemia. This phenomenon has been termed myocardial preconditioning and is believed to be partially attributable to the release of adenosine during the preconditioning period.

Other studies have shown that adenosine and adenosine analogues reduce the extent of tissue damage that is observed following the interruption of blood flow to the heart in a variety of models of ischemic injury in several species (see, for example, Toombs, C. et al., "Myocardial protective effects of adenosine. Infarct size reduction with pretreatment and continued receptor stimulation during ischemia.", *Circulation* 86, 986–994 (1992); Thornton, J. et al., "Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction.", *Circulation* 85, 659–665 (1992); and Downey, J., "Ischemic preconditioning—nature's own cardioprotective intervention.", *Trends Cardiovasc. Med.* 2(5), 170–176 (1992)).

Compounds of the present invention mimic myocardial preconditioning, thereby ameliorating ischemic injury or producing a reduction in the size of myocardial infarct consequent to myocardial ischemia and are thereby useful as cardioprotective agents.

Antilipolysis

Hyperlipidemia and hypercholesterolemia are known to be two of the prime risk factors for atherosclerosis and coronary heart disease, the leading cause of death and disability in Western countries. Although the etiology of atherosclerosis is multifactorial, the development of atherosclerosis and conditions including coronary artery disease, peripheral vascular idsease and cerbrovascular disease resulting from restricted blood flow, are associated with abnormalities in serum cholesterol and lipid levels. The etiology of hypercholesterolemia and hyperlipidemia is primarily genetic, although factors such as dietary intake of saturated fats and cholesterol may contribute.

The antilipolytic activity of adenosine and adenosine analogues arises from the activation of the A_1 receptor subtype (Lohse, M. J., et al., *Recent Advances in Receptor Chemistry*, Melchiorre, C. and Gianella, Eds, Elsevier Science Publishers B.V., Amsterdam, 1988, 107–121). Stimulation of this receptor subtype lowers the intracellular cyclic AMP concentration in adipocytes. Cyclic AMP is a necessary co-factor for the enzyme lipoprotein lipase which hydrolytically cleaves triglycerides to free fatty acids and glycerol in adipocytes (Egan, J. J., et al., *Proc. Natl. Acad. Sci.* 1992 (89), 8357–8541). Accordingly, reduction of intracellular cyclic AMP concentration in adipocytes reduces lipoprotein lipase activity and, therefore, the hydrolysis of triglycerides.

Elevated blood pressure and plasma lipids, including triglycerides, are two well accepted risk factors associated with mortality resulting from cardiovascular disease.

For the diabetic patient, where the likelihood of mortality from cardiovascular disease is substantially greater, the risk associated with these factors is further magnified (Bierman, E. L., *Arteriosclerosis and Thrombois* 1992 (12), 647–656). Additionally, data suggest that excessive lipolysis is characteristic of non-insulin dependent diabetes and possibly contributes to insulin resistance and hyperglycemia (Swislocki, A. L., *Horm. Metab. Res.* 1993 (25), 90–95).

Compounds of the present invention, as antihypertensive and antilipolytic agents, are useful in the treatment and amelioration of both vascular and metabolic risk factors, and are of particular value and utility.

The present invention relates to a class of adenosine analogues and their utility in the treatment of hypertension, myocardial ischemia, as cardioprotective agents which ameliorate ischemic injury or myocardial infarct size consequent to myocardial ischemia, and as antilipolytic agents which reduce plasma lipid levels, serum triglyceride levels, and plasma cholesterol levels, and to methods and intermediates used in the preparation of such compounds.

2. Reported Developments

Adenosine has a wide variety of physiological and pharmacological actions including a marked alteration of cardiovascular and renal function. In animals and man, intravenous injection of the adenosine nucleotide causes hypotension.

The physiological and pharmacological actions of adenosine are mediated through specific receptors located on cell surfaces. Four adenosine receptor subtypes, designated as A1, A_{2A}, A_{2B}, and A_3 receptors, have been identified. The A_1 receptor inhibits the formation of cAMP by suppressing the activity of adenylate cyclase, while stimulation of A_2 receptors increases adenylate cyclase activity and intracellular cAMP. Each receptor appears to mediate specific actions of adenosine in different tissues: for example, the vascular actions of adenosine appears to be mediated through stimulation of A_2 receptors, which is supported by the positive correlation between cAMP generation and vasorelaxation in adenosine-treated isolated vascular smooth muscle; while stimulation of the cardiac A_1 receptors reduces cAMP generation in the heart which contributes to negative dromotropic, inotropic and chronotropic cardiac effects. Consequently, unlike most vasodilators, adenosine administration does not produce a reflex tachycardia.

Adenosine also exerts a marked influence on renal function. Intrarenal infusion of adenosine causes a transient fall in renal blood flow and an increase in renal vascular resistance. With continued infusion of adenosine, renal blood flow returns to control levels and renal vascular resistance is reduced. The initial renal vasoconstrictor responses to adenosine are not due to direct vasoconstrictor actions of the nucleotide, but involve an interaction between adenosine and the renin-angiotensin system.

Adenosine is widely regarded as the primary physiological mediator of reactive hyperemia and autoregulation of the coronary bed in response to myocardial ischemia. It has been reported that the coronary endothelium possesses adenosine A_2 receptors linked to adenylate cyclase, which are activated in parallel with increases in coronary flow and that cardiomyocyte receptors are predominantly of the adenosine A_1 subtype and associated with bradycardia. Accordingly, adenosine offers a unique mechanism of ischemic therapy.

Cardiovascular responses to adenosine are short-lived due to the rapid uptake and metabolism of the endogenous nucleotide. In contrast, the adenosine analogues are more resistant to metabolic degradation and are reported to elicit sustained alterations in arterial pressure and heart rate.

Several potent metabolically-stable analogues of adenosine have been synthesized which demonstrate varying degrees of selectivity for the two receptor subtypes. Adenosine agonists have generally shown greater selectivity for A_1 receptors as compared to A_2 receptors. Cyclopentyladenosine (CPA) and R-phenylisopropyl-adenosine (R-PIA) are standard adenosine agonists which show marked selectivity for the A_1 receptor (A_2/A_1 ratio=780 and 106, respectively). In contrast, N-5'-ethyl-carboxamido adenosine (NECA) is a potent A_2 receptor agonist (Ki--12 nM) but has equal affinity for the A_1 receptor (Ki-6.3 nM; A_2/A_1 ratio=1.87). Until recently, CV-1808 was the most selective A_2 agonist available (A_2/A_1=0.19), even though the compound was 10-fold less potent than NECA in its affinity for the A_2 receptor. In recent developments, newer compounds have been disclosed which are very potent and selective A_2 agonists (Ki=3–8 nM for A_1; A_2/A_1 ratio=0.027–0.042) (C. E. Müller and T. Scior, *Pharmaceutica Aca Hevetiae* 68 (1993) 77–111).

Various N6-aryl and N6-heteroarylalkyl substituted adenosines, and substituted-(2-amino and 2-hydroxy) adenosines, have been reported in the literature as possessing varied pharmacological activity, including cardiac and circulatory activity. See, for example, British Patent Specification 1,123,245, German Offen. 2,136,624, German Off 2,059,922, German Offen. 2,514,284, South African Patent No. 67/7630, U.S. Pat. No. 4,501,735, EP Publication No. 0139358 (disclosing N6-[geminal diaryl substiuted alkyl] adenosines), EP Patent Application Ser. No. 88106818.3 (disclosing that N6-heterocyclic-substituted adenosine derivatives exhibit cardiac vasodilatory activity), German Offen. 2,131,938 (disclosing aryl and heteroaryl alkyl hydrazinyl adenosine derivatives), German Offen. 2,151,013 (disclosing N6-aryl and heteroaryl substituted adenosines), German Offen. 2,205,002 (disclosing adenosines with N6-substituents comprising bridged ring structures linking the N6-nitrogen to substituents including thienyl) and South African Patent No. 68/5477 (disclosing N6-indolyl substituted-2-hydroxy adenosines).

U.S. Pat. No. 4,954,504 and EP Publication No. 0267878 disclose generically that carbocyclic ribose analogueues of adenosine, and pharmaceutically acceptable esters thereof, substituted in the 2- and/or N6- positions by aryl lower alkyl groups including thienyl, tetrahydropyranyl, tetrahydrothiopyranyl, and bicyclic benzo fused 5- or 6-membered saturated heterocyclic lower alkyl derivatives exhibit adenosine receptor agonist properties. Adenosine analogueues having thienyl-type substituents are described in EP Publication No. 0277917 (disclosing N6-substituted-2-heteroarylalkylamino substituted adenosines including 2-[(2-[thien-2-yl]ethyl)amino]substituted adenosine), German Offen. 2,139,107 (disclosing N6-[benzothienylmethyl]-adenosine), PCT WO 85/04882 (disclosing that N6-heterocyclicalkyl-substituted adenosine derivatives, including N6-[2-(2-thienyl)ethyl]amino-9-(D-ribofuranosyl)9H-purine, exhibit cardiovascular vasodilatory activity and that N6-chiral substituents exhibit enhanced activity), EP Published Application No. 0232813 (disclosing that N6-(1-substituted thienyl) cyclopropylmethyl substituted adenosines exhibit cardiovascular activity), U.S. Pat. No. 4,683,223 (disclosing that N6-benzothiopyranyl substituted adenosines exhibit antihypertensive properties), PCT WO 88/03147 and WO 88/03148 (disclosing that N6-[2-aryl-2-(thien-2-yl)]ethyl substituted adensosines exhibit antihypertensive properties), U.S. Pat. Nos. 4,636,493 and 4,600,707 (disclosing that N6-benzothienylethyl substituted adenosines exhibit antihypertensive properties).

Adenosine-5'-carboxylic acid amides are disclosed as having utility as anti-hypertensive and anti-anginal agents in U.S. Pat. No. 3,914,415, while U.S. Pat. No. 4,738,954 discloses that N6-substituted aryl and arylalkyl-adenosine 5'-ethyl carboxamides exhibit various cardiac and antihypertensive properties.

N^6-alkyl-2'-O-alkyl adenosines are disclosed in EP Publication No. 0,378,518 and UK Patent Application No. 2,226,027 as having antihypertensive activity. N^6-alkyl-2', 3'-di-O-alkyl adenosines are also reported to have utility as antihypertensive agents, U.S. Pat. No. 4,843,066.

Adenosine-5'-(N-substituted)carboxamides and carboxylate esters and N1-oxides thereof are reported to be coronary vasodilators, Stein, et al., *J. Med. Chem.* 1980, 23, 313–319 and *J. Med. Chem.* 19 (10), 1180 (1976). Adenosine-5'-carboxamides and N1-oxides thereof are also reported as small animal poisons in U.S. Pat. No. 4,167,565.

The antilipolytic activity of adenosine is described by Dole, V. P., *J. Biol. Chem.* 236 (12), 3125–3130 (1961). Inhibition of lipolysis by (R)- N^6 phenylisopropyl adenosine is disclosed by Westermann, E., et al., *Adipose Tissue, Regulation and Metabolic Functions*, Jeanrenaud, B. and Hepp, D. Eds., George Thieme, Stuttgart, 47–54 (1970). N^6-mono- and disubstituted adenosine analogueues are disclosed as having antilipolytic, antihypercholesterolemic, and antilhyperlipemic activity in U.S. Pat. Nos. 3,787,391; 3,817,981; 3,838,147; 3,840,521; 3,835,035; 3,851,056; 3,880,829; 3,929,763; 3,929,764; 3,988,317; and 5,032,583.

N6-substituted adenosines and analogues, useful in treating gastroinstestinal motility disorders, have been reported in EP Published Applications Nos. 0423776, and 0423777.

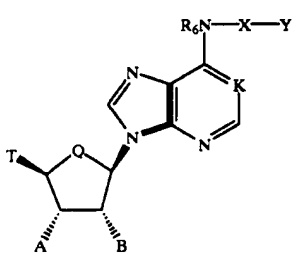
N6-heterocyclyl compounds derived from adenosine and analogues thereof, and their use in treating hypertension and myocardial ischemia, their use as cardioprotective agents which ameliorate ischemic injury or myocardial infarct size consequent to myocardial ischemia, their use as antilipolytic agents which reduce plasma lipid levels, serum triglyceride levels, and plasma cholesterol levels, are disclosed in U.S. patent application Ser. No. 08/316,761, filed Oct. 3, 1994, assigned to the same assignee as the present application, and for which a Notice of Allowance was mailed Mar. 26, 1996. N6-heterocyclyl compounds derived from adenosine and analogues thereof, and their use in treating myocardial ischemia and hypertension, are also disclosed in U.S. Pat. No. 5,364,862, filed Oct. 2, 1992, and which is assigned to the same assignee as the present application.

It is believed that the reported toxicity, CNS properties and heart rate elevation associated with adenosine analogueues have contributed to the difficulties preventing the development of a commercial adenosine analogue antihypertensive/antiischemic agent. The present invention relates to a class of metabolically stable adenosine analogues, and derivatives thereof, possessing unexpectedly desirable pharmacological properties, i.e. are antihypertensive, cardioprotective, anti-ischemic, and antilipolytic agents having a unique therapeutic profile.

SUMMARY OF THE INVENTION

The compounds of the present invention are described by Formula I

Formula I

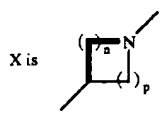
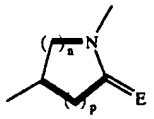


wherein:

K is N, NO, or CH;

Q is CH_2 or O;

R_6 is hydrogen, alkyl, allyl, 2-methylallyl, 2-butenyl, or cycloalkyl;

X is  or 

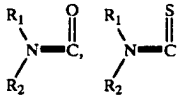
where the nitrogen of the ring of X is substituted by Y;

E is O or S;

Y is hydrogen, alkyl, aralkyl, substituted aralkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heterocyclylalkyl, or substituted heterocyclylalkyl;

n and p are independently 0, 1, 2, or 3, provided at n+p is at least 1;

T is hydrogen, alkyl, acyl, thioacyl, halo, carboxyl,



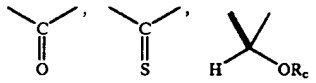
or $R_3O—CH_2$;

R_1, R_2, and R_3 are independently H, alkyl, or cycloalkyl;

A is hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, or OR';

B is hydrogen, alkyl, hydroxyalkyl, alkoxyalkyl, or OR";

R' and R" are independently hydrogen, alkyl, aralkyl, carbamoyl, alkyl carbamoyl, dialkylcarbamoyl, acyl, alkoxycarbonyl, aralkoxycarbonyl, aryloxycarbonyl, or, when A and B are OR' and OR", respectively, R' and R" together may form



where R_c is hydrogen or alkyl

where R_d and R_e are independently hydrogen, alkyl, or together with the carbon atom to which they are attached may form a 1,1-cycloalkyl group;

or a pharmaceutically acceptable salt thereof, pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

This invention relates also to methods for treating cardiovascular disease marked by hypertension or myocardial ischemia using pharmaceutical compositions including an anti-hypertensive effective amount or an anti-ischemic effective amount of a compound of Formula I above, to a method for ameliorating ischemic injury or myocardial infarct size using pharmaceutical compositions including a cardioprotective amount of a compound of Formula I above, to a method for treating hyperlipidemia or hypercholesterolemia using pharmaceutical compositions including an antilipolytic amount of Formula I, and to methods and intermediates used in the preparation of such compounds.

DETAILED DESCRIPTION

As used above and throughout the description of the invention, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Acyl" means a straight or branched alkyl-C=O group. "Thioacyl" means a straight or branched alkyl-C=S group. Preferred acyl and thioacyl groups are lower alkanoyl and lower thioalkanoyl having from 1 to about 6 carbon atoms in the alkyl group.

"Alkyl" means a saturated aliphatic hydrocarbon group which may be straight or branched and having about 1 to about 20 carbon atoms in the chain. Preferred alky groups may be straight or branched and have about 1 to about 10 carbon atoms in the chain. Branched means that a lower alkyl group such as methyl, ethyl or propyl is attached to a linear alkyl chain.

"Lower alkyl" means an alkyl group having 1 to about 6 carbons.

"Cycloalkyl" means an aliphatic ring having 3 to about 10 carbon atoms in the ring. Preferred cycloalkyl groups have 4 to about 7 carbon atoms in the ring.

"Carbamoyl" means an

group. Alkylcarbamoyl and dialkylcarbamoyl means that the nitrogen of the carbamoyl is substituted by one or two alkyl groups, respectively.

"Carboxyl" means a COOH group.

"Alkoxy" means an alkyl-O group in which "alkyl" is as previously described. Lower alkoxy groups are preferred. Exemplary groups include methoxy, ethoxy, n-propoxy, i-propoxy and n-butoxy.

"Alkoxyalkyl" means an alkyl group, as previously described, substituted by an alkoxy group, as previously described.

"Alkoxycarbonyl means an alkoxy-C=O group.

"Aralkyl" means an alkyl group substituted by an aryl radical, wherein "aryl" means a phenyl or naphthyl. "Substituted aralkyl" and "substituted aryl" means that the aryl group, or the aryl group of the aralkyl group is substituted with one or more substituents which include alkyl, alkoxy, amino, nitro, carboxy, carboalkoxy, cyano, alkyl amino, halo, hydroxy, hydroxyalkyl, mercaptyl, alkylmercaptyl, trihaloalkyl, carboxyalkyl or carbamoyl.

"Aralkoxycarbonyl" means an aralkyl—O—C=O group.

"Aryloxycarbonyl" means an aryl—O—C=O group.

"Carbalkoxy" means a carboxyl substituent esterified with an alcohol of the formula $C_nH_{2n+1}OH$, wherein n is from 1 to about 6.

"Halogen" (or "halo") means chlorine (chloro), fluorine (fluoro), bromine (bromo) or iodine (iodo).

"Heterocyclyl" means about a 4 to about a 10 membered ring structure in which one or more of the atoms in the ring is an element other than carbon, e.g., N, O or S. Heterocyclyl may be aromatic or non-aromatic, i.e., may be saturated, partially or fully unsaturated.

Preferred heterocyclyl groups include pyridyl, pyridazinyl, pyrimidinyl, isoquinolinyl, quinolinyl, quinazolinyl, imidazolyl, pyrrolyl, furanyl, thienyl, thiazolyl, benzothiazolyl, piperidinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydropyranyl, and morphonlinyl groups.

"Substituted heterocyclyl" means that the heterocyclyl group is substituted by one or more substituents wherein the substituents include alkoxy, alkylamino, aryl, carbalkoxy, carbamoyl, cyano, halo, heterocyclyl, trihalomethyl, hydroxy, mercaptyl, alkylmercaptyl or nitro.

"Hydroxyalkyl" means an alkyl group substituted by a hydroxy group. Hydroxy lower alkyl groups are preferred. Exemplary preferred groups include hydroxymethyl, 2-hydroxymethyl, 2-hydroxypropyl and 3-hydroxypropyl.

"Prodrug" means a compound which is rapidly transformed in vivo to yield the parent peptide compound, for example by hydrolysis in blood. "Pharmaceutically acceptable prodrug" means a compound which is, within the scope of sound medical judgement, suitable for pharmaceutical use in a patient without undue toxicity, irritation, allergic response, and the like, and effective for the intended use, including a pharmaceutically acceptable ester as well as a zwitterionic form, where possible, of the peptide compounds of the invention. Pharmaceutically acceptable prodrugs according to the invention are described in T. Higuchi and V. Stella, *Pro-drugs as Novel Delivery Systems*, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., *Bioreversible Carriers in Drug Design*, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

"Solvate" means a physical association of a compound of this invention with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Representative solvates include ethanolates, methanolates, and the like. "Hydrate" is a solvate wherein the solvent molecule(s) is/are H_2O.

"Cardioprotection" refers to the effect whereby the myocardium is made less susceptible to ischemic injury and myocardial infarct consequent to myocardial ischemia.

"Amelioration of ischemic injury" means the prevention or reduction of ischemic injury to the myocardium consequent to myocardial ischemia.

"Amelioration of myocardial infarct size" means the reduction of the myocardial infarct size, or the prevention of myocardial infarct, consequent to myocardial ischemia.

The compounds of Formula I contain chiral (asymmetric) centers. The invention includes the individual stereoisomers and mixtures thereof. The individual isomers are prepared or isolated by methods well known in the art or by methods described herein.

The compounds of the invention may be used in the form of the free base, in the form of acid addition salts or as hydrates. All such forms are within the scope of the invention. Acid addition salts are simply a more convenient form for use. In practice, use of the salt form inherently amounts to use of the base form. The acids which may be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the recipient in pharmaceutical doses of the salts, so that the beneficial anti-hypertensive, cardioprotective, anti-ischemic, and antilipolytic effects produced by the free base are not vitiated by side effects

ascribable to the anions. Although pharamaceutically acceptable salts of the compounds of the invention are preferred, all acid addition salts are useful as sources of the free base form, even if the particular salt, per se, is desired only as an intermediate product as, for example, when the salt is formed only for purposes of purification and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures. Pharmaceutically acceptable salts within the scope of the invention are those derived from the following acids: mineral acids such as hydrochloric acid, sulfuric acid, phosphoric acid, and sulfamic acid; and organic acids such as acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, fumaric acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, quinic acid and the like. The corresponding acid addition salts comprise the following: hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartarate, methanesulfonate, fumarate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfonate and quinate, respectively.

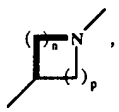
The acid addition salts of the compounds of the invention are conveniently prepared either by dissolving the free base in aqueous or aqueous-alcohol solution or other suitable solvents containing the appropriate acid and isolating the salt by evaporating the solution, or by reacting the free base and acid in an organic solvent, in which case the salt separates directly or can be obtained by concentration of the solution.

Included within the scope of Formula I are classes of compounds which may be characterized generally as N6 -substituted adenosines; N6-substituted carbocyclic adenosines (or, alternatively, dihydroxy[N6-substituted-9-adenyl] cyclopentanes) and N-oxides thereof; and N6-substituted-N'-1-deazaaristeromycins (or, alternatively, dihydroxy[N7-substituted[4,5-b]imidazopyridyl]-cyclopentanes). Also within the scope of Formula I are the 5'-alkylcarboxamide derivatives of the adenosines, the carbocyclic adenosines and the 1-deazaaristeromycins, the derivatives of compounds of the above classes in which one or both of the 2- or 3- hydroxyl groups of the cyclopentane ring or, in the cases of classes of compounds containing the ribose moiety, the 2'- or 3'- hydroxyl groups of the ribose ring are substituted. Such derivatives may themselves comprise the biologically active chemical entity useful in the treatment of hypertension and myocardial ischemia, and as cardioprotective and antilipolytic agents, or may act as pro-drugs to such biologically active compounds which are formed therefrom under physiological conditions.

Representative compounds of the invention include: (2R, 3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-chloropyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3, 4-diol, (2R,3S,4R,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(R)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R) 2-hydroxymethyl-5-[6-[1-(5-bromopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-(6-(1-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino)-purin-9-yl) tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(5'-trifluoromethyl-3,4,5,6-tetrahydro-2H-[1,2']-bipyridinyl-3-yl)-purin-9-yl]tetrahydrofuran-3,4-diol, (2R, 3R,4S,5R)-2-hydroxymethyl-5-[6-(phenylpyrrolidin-3(S)-ylamino)-purin-9-yl(tetrahydrofuran-3,4-diol, (2R,3R,4S, 5R)-2-hydroxymethyl-5-[6-(1-pyridin-2-ylpyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S, 5R)-2-hydroxymethyl-5-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R, 3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3, 4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-thiophen-2-ylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylmercaptopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R, 3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (1S,2R,3S,4R)-2,3-dihydroxy-4-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3-ylamino]-purin-9-yl]cyclopentanecarboxylic acid ethylamide, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)piperidin-4-yl]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3S)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol dihydrochloride, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(R)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3R)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4(R)-1-benzyl-4-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino] pyrrolidin-2-one hydrochloride, (1R,2S,3R,5S)-5-methyl-3-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S, 3R,5R)-5-[6-[1-(5-chloropyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(pyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4(S)-1-benzyl-4-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino]pyrrolidin-2-one hydrochloride, (1R,2S, 3R,5R)-3-hydroxymethyl-5-[6-[1-(quinolin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S, 3R,5R)-3-hydroxymethyl-5-[6-[1-S-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4,5-bistrifluorpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(phenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4-[3(S)-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-

9H-purin-6-ylamino]pyrrolidin- 1-yl]benzonitrile, (1R,2S, 3R,5R)-3-hydroxymethyl-5-[6-[1-(isoquinolin-1-yl) pyrrolidin-3(S)ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-bromoquinolin-2-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(4-chlorophenyl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-3-[6-[1-(3-chloro-5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R, 2S,3R,5R)-3-isopropoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-isopropoxymethyl-5-[6-[1-(4-trifluoromethylpyridin2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R, 5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S, 3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chloropyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylphenyl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S) ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-phenylpyrrolidin-3-(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino) purin-9-yl]5-methoxymethylcyclopentane-1,2-diol, 5'-N-[1 (S)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl]carbocyclic adenosine-5'-uronamide, and 5'-N-[1(R)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3-(S)-yl]carbocyclic adenosine-5'-uronamide.

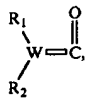
A preferred class of compounds of the invention is described by Formula I wherein K is N, T is hydroxymethyl or methoxymethyl, A and B are hydroxy, X is



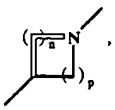
and n+p is 3 or 4, or pharmaceutically acceptable salts thereof. Representative compounds of this preferred class of compounds include (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-chloropyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3S,4R,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(R)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)2-hydroxymethyl-5-[6-[1-(5-bromopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-(6-(1-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino)-purin-9-yl) tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(5'-trifluoromethyl-3,4,5,6-tetrahydro-2H-[1,2']-bipyridinyl-3-yl)-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(phenylpyrrolidin-3(S)-ylamino)-purin-9-yl] tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(1-pyridin-2-ylpyrrolidin-3(S)-ylamino]-purin-9-yl] tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylpyridin-2-yl)-pyrrolidin-3 (S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R, 4S,5R)-2-hydroxymethyl-5-[6-[1-(5-thiophen-2-ylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylmercaptopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-_pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)piperidin-4-yl]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3S)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol dihydrochloride, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(R)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3R)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chloropyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(pyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(quinolin-3-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-S-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4,5-

bistrifluorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(phenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4-[3(S)-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino]pyrrolidin-1-yl]benzonitrile, (1R,2S, 3R,5R)-3-hydroxymethyl-5-[6-[1-(isoquinolin-1-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-bromoquinolin-2-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(4-chlorophenyl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-3-[6-[1-(3-chloro-5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R, 2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R, 5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S, 3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chlorpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylphenyl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-phenylpyrrolidin-3-(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-hydroxymethylcyclopentane-1,2-diol, and (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-methoxymethylcyclopentane-1,2-diol.

Another preferred class of compounds of the invention is described by Formula I wherein Q is CH_2, K is N, T is

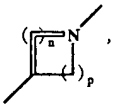


wherein R_1 is H and R_2 is lower alkyl, A and B are hydroxy, X is



and n+p is 3 or 4, or pharmaceutically acceptable salts thereof. Representative compounds of this other preferred class of compounds include, (1S,2R,3S,4R)-2,3-dihydroxy-4-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentanecarboxylic acid ethylamide, 5'-N-[1(S)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl]carbocyclic adenosine-5'-uronamide, and 5'-N-[1(R)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl] carbocyclic adenosine-5'-uronamide.

A more preferred class of compounds of the invention is described by Formula I wherein Q is CH_2, K is N, T is hydroxymethyl or methoxymethyl, A and B are hydroxy, X is



and n+p is 3 or 4, or pharmaceutically acceptable salts thereof. Representative compounds of this more preferred class of compounds include (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)piperidin-4-yl]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3S)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol dihydrochloride, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(R)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3R)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chloropyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(pyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(quinolin-3-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-S-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4,5-bistrifluorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(phenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4-[3(S)-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino]pyrrolidin-1-yl]benzonitrile, (1R,2S, 3R,5R)-3-hydroxymethyl-5-[6-[1-(isoquinolin-1-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol,

(1R,2S,3R,5R)-5-[6-[1-(6-bromoquinolin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4-chlorophenyl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-[1-(3-chloro-5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2diol, (1R,2S,3R,5R)-5-[6-[1-(5-chlorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylphenyl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-phenylpyrrolidin-3-(S)-ylamino]-purin-9-yl]cyclopentan-1,2-diol, (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-hydroxymethylcyclopentane-1,2-diol, and (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-methoxymethylcyclopentane-1,2-diol.

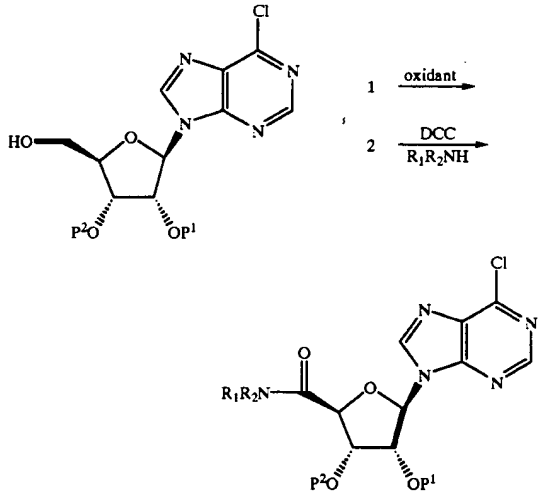
Most preferred compound of the present invention include (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol and (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol.

Compounds of this invention may be prepared by known methods or in accordance with the reaction sequences described below. The starting materials used in the preparation of compounds of the invention are known or commercially available, or can be prepared by known methods or by specific reaction schemes described herein.

Compounds of Formula I, wherein K is N, Q is O and T is $R_3O—CH_2$, may be prepared by reacting commercially-available 6-chloropurine riboside with various unsubstituted, alkyl, aralkyl, aryl, substituted aryl, heterocyclyl, or substituted heterocyclyl azacycloalkylamines or protected derivatives thereof (hereinafter, "appropriate starting amines") as exemplified below.

Compounds of Formula I, wherein K is N. Q is O and T is $R_1R_2N—C=O$ are similarly prepared starting with the product of Reaction Scheme A. In this reaction, 6-chloropurine riboside, with the 2'- and 3'- hydroxyl groups of the ribose ring protected, is treated with an oxidant, for example a Jones reagent, and the product acid treated with either dicyclohexlcarbodiimide (DCC) or BOP-Cl in the presence of a selected amine, to yield the 5'-alkylcarboxamide derivative.

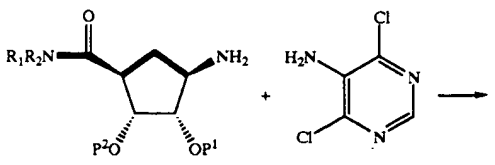
REACTION SCHEME A



(P = protecting group)

Suitable starting materials for compounds of Formula I wherein K is N, Q is CH_2 and T is $R_1R_2N—C=0$, may be prepared as described by Chen et al., Tetrahedron Letters 30: 5543–46(1989). Alternatively, Reaction Scheme B may be used to prepare such starting materials. In carrying out Reaction Scheme B, the 4-ethylcarboxamide derivative of 2,3-dihydroxycyclopentylamine, prepared as described by Chen et al., is reacted with 3-amino-2,4-dichloropyrimidine. The product of this initial reaction is then heated with an aldehydylamidine acetate, for example formamidine acetate in dioxane and methoxyethanol, for a time sufficient to effect ring closure (from about 30 min to about 4 hours), thereby yielding a product which may be conveniently reacted with appropriate starting amines in the manner described below, to give the compounds of the invention. The order of reaction is not critical. For example, the intermediate formed in Reaction Scheme B could be reacted with an appropriate starting amine, followed by ring closure to yield the desired final product.

REACTION SCHEME B



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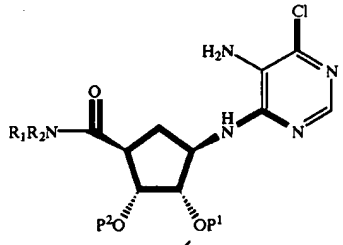
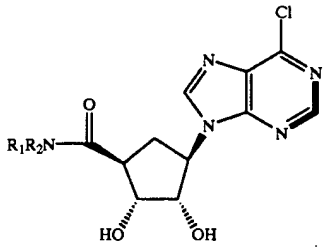


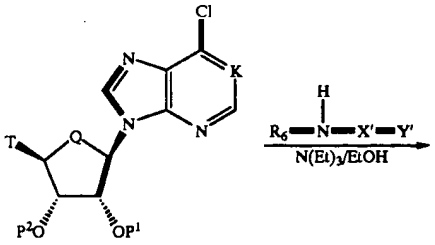


Various amines, useful in forming the compounds of this invention, may be prepared by methods known in the art, or by methods described herein.

Diastereomeric mixtures of compounds or intermediates useful in preparing compounds of the present may be separated into single racemic or optically active enantiomers by methods known in the art; for example, by chromatography, fractional distillation or fractional crystallization of d- or l-(tartarate, dibenzoyltartarate, mandelate or camphorsulfonate) salts.

The N6-substituted adenosines and carbocyclic adenosines of the invention may be formed by reacting 6-chloropurine riboside or the products of Reaction Schemes A or B with various appropriate starting amines, as exemplified in Reaction Scheme C.

REACTION SCHEME C



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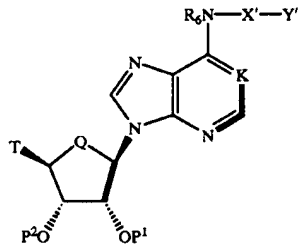
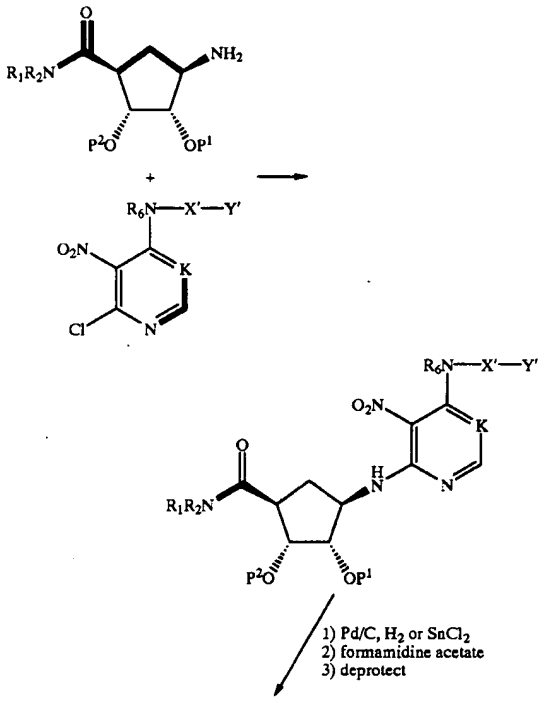


Where X' and Y' are X and Y as defined hereinabove, or protected derivatives thereof.

The N6 -substituted-N'alkyl-deazaaristeromycins of the invention may be prepared as shown in Reaction Scheme D.

REACTION SCHEME D



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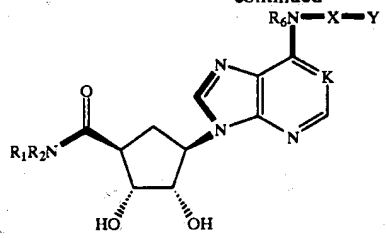
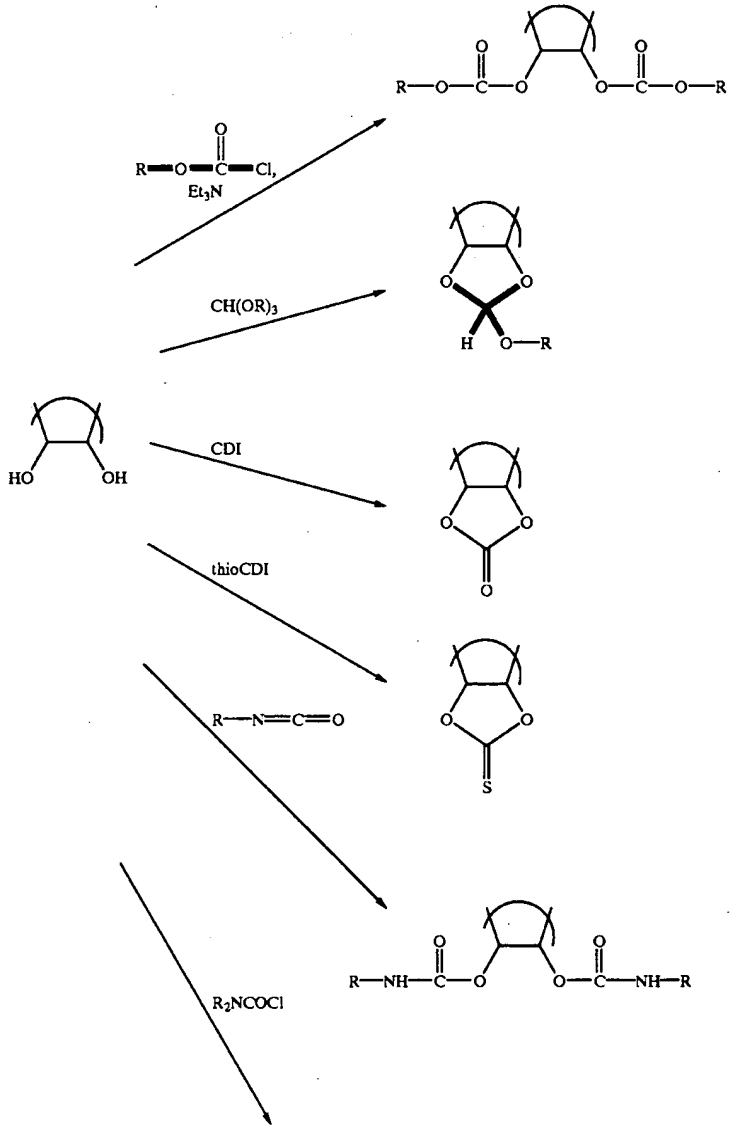


Compounds of the present invention which may act as pro-drugs include those compounds wherein the hydroxyl groups on the ribose or cyclopentane ring are substituted with groups R' and R" as defined above for Formula I. These may be prepared by known methods and are exemplified by the preparations shown in Reaction Scheme E, below.

REACTION SCHEME E



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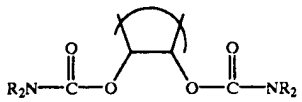


Treatment of the dihydroxy compounds with a chloroformate ester in the presence of an organic base, for example triethylamine, will give the corresponding bis-carbonate. The alkoxymethylene acetal may be prepared by treatment with the corresponding orthoester in the presence of a catalytic amount of p-toluenesulfonic acid. The carbonate is available by treatment with 1,1'-carbonyldiimidazole and the thiocarbonate by treatment with thiocarbonyldiimidizole. The alkyl and dialkylcarbamoyl derivatives may be prepared by treatment with the corresponding alkyl isocyanate or dialkyl carbamoyl chloride in the presence of an organic base respectively.

Compounds of the present invention wherein K is an N-oxides, may be prepared by oxidation of the corresponding adenosine or carbocyclic adenosine by known methods, for example by treatment with hydrogen peroxide in acetic acid.

The 2'-O-alkyl derivatives may be prepared by known methods, for example by reaction of the appropriate starting amine with 6-chloro-9-(2'-O-methyl-b-D-ribofuranosyl)-9H-purine.

Functional groups of starting compounds and intermediates that are used to prepare the compounds of the invention may be protected by common protecting groups known in the art. Conventional protecting groups for amino and hydroxyl functional groups are described, for example, in T. W. Greene, "Protective Groups in Organic Synthesis", Wiley, New York (1984).

Hydroxyl groups may be protected as esters, such as acyl derivatives, or in the form of ethers. Hydroxyl groups on adjacent carbon atoms may advantageously be protected in the form of ketals or acetals. In practice, the adjacent 2' and 3' hydroxyl groups of the starting compounds in Reaction Schemes A and B are conveniently protected by forming the 2',3' isopropylidene derivatives. The free hydroxyls may be restored by acid hydrolysis, for example, or other solvolysis or hydrogenolysis reactions commonly used in organic chemistry.

Following synthesis, compounds of the invention are typically purified by medium pressure liquid chromatography (MPLC), on a chromatotron, radially accelerated thin layer chromatography, flash chromatography or column chromatography through a silica gel or Florisil matrix, followed by crystallization. For compounds of Formula I wherein K is N, Q is O and T is $R_3O—CH_2$, typical solvent systems include chloroform:methanol, ethyl acetate:hexane, and methylene chloride:methanol. Eluates may be crystallized from methanol, ethanol, ethyl acetate, hexane or chloroform, etc.

For compounds of Formula I, wherein K is N, Q is O, and T is $R_1R_2N—C=O$, typical solvent systems include chloroform:methanol. For example, eluates may be crystallized from 50–100% ethanol (aqueous).

For compounds of Formula I, wherein Q is CH_2, K is N or CH, and T is $R_1R_2N—C=O$, typical solvent systems include methylene chloride:methanol. For example, eluates may be crystallized from ethyl acetate with or without methanol, ethanol or hexane.

Compounds requiring neutralization may be neutralized with a mild base such as sodium bicarbonate, followed by washing with methylene chloride and brine. Products which are purified as oils are sometimes triturated with hexane/ethanol prior to final crystallization.

The method of the present invention is further illustrated and explained by the following Examples.

EXAMPLE 1

Preparation of 5'-N-Ethyl-2',3'-isopropylidene- N^6-chloroadenosine-5'-uronamide

Step 1: N^6-Chloro-2',3'-isopropylideneadenosine

6-Chloropurine riboside (31.5 g), triethylorthoformate (73 mL) and TsOH (19.8 g) are stirred in 600 mL acetone for 2 hours at RT. The reaction mixture is concentrated in vacuo, combined with ethyl acetate and washed with saturated $NaHCO_3$ solution, and brine, dried (Na_2SO_4) and concentrated to yield N^6-Chloro-2',3'-isopropylideneadenosine as a white solid.

Step 2: N^6-Chloro-2',3'-Isopropylideneadenosine-5'-carboxylic acid

N^6-Chloro-2',3'-isopropylideneadenosine (4.5 g, 13.8 mmol) and 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy benzoate (4-hydroxy-TEMPO benzoate) (0.0381 g, 0.14 mmol) are combined in acetonitrile, 5% $NaHCO_3$ (87%) is added to the reaction mixture and sodium bromite hydrate (10.41 g, 55.1 mmol) is added portionwise at 0–5° C. The reaction mixture is then allowed to warm to room temperature, and the solution was stirred vigorously for about 3 hours. 10% tartaric acid solution is added and the aqueous layer is separated and extracted with ethyl acetate (3×). The combined organic layers are washed with 5% sodium bicarbonate solution (3×). The basic layers are combined and reacidified to pH 3 with concentrated hydrochloric acid. The aqueous layers are extracted with ethyl acetate (3×). The combined organic layers are then washed with brine and dried over magnesium sulfate. The filtrate is concentrated to an amorphous white solid, co-evaporated with 3 portions of toluene and dried in vacuo to give N^6-chloro-2',3'-isopropylideneadenosine-5'-carboxylic acid.

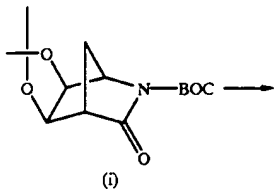
Step 3: 5'-N-Ethyl-2',3'-isopropylidene-N^6-chloroadenosine-5'-uronamide

N^6-chloro-2',3'-isopropylideneadenosine-5'-carboxylic acid (4.4 g, 12.9 mmol), triethylamine (1.64 mL, 11.7 mmol) isopropenyl chloroformate (1.28 mL, 11.7 mmol), and methylene chloride (50 mL) are combined under argon at −10° C. and stirred for about 2 minutes. Ethylamine (0.77 mL, 11.7 mmol) is added to the reaction mixture and stirring continued for an additional 1 minute. The reaction mixture is partitioned between methylene chloride and saturated sodium bicarbonate. The aqueous layers are washed with methylene chloride (3×). The combined organic layers are washed with brine and dried over sodium sulfate, filtered, evaporated in vacuo and the residue purified by flash chromatography on silica gel, eluting with 3% MeOH/$CHCl_3$, to give 5'-N-ethyl-2',3'-isopropylidene-N^6-chloroadenosine-5'-uronamide, 1H NMR (300 MHz, (CDCl3) d 8.75 (s, 1H), 8.23 (s, 1H), 6.20 (d, 1H), 5.50 (dd, 2H), 4.73 (d, 1H), 3.01 (m, 2H), 1.63 (s, 1H), 1.41 (s, 3H), 0.77 (t, 3H).

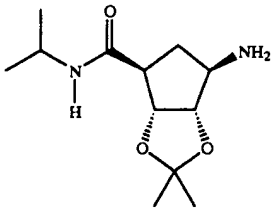
EXAMPLE 2

Preparation of (1S,2R,3S,4R)-2,3-dihydroxy-4-[6-[1-(4-trifluormethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]purin-9-yl]cyclopentanecarboxylic acid isopropylamide

Step (1)



(i)

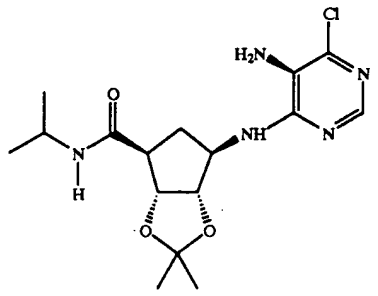


(ii)

15.5 g (54.6 mmol) N-BOC-5,6-Dimethylenedioxy-2-azabicyclo[2.2.1]heptan-3-one (i) (prepared as in Step (6) of Example 3, below) is dissolved in 16 mL isopropyl amine and the mixture stirred at room temperature for about 2 hours. The mixture is evaporated in vacuo, and the residue azeotroped with chloroform to give a white solid. This solid is dissolved in 250 mL ethyl acetate, the solution cooled to 0° C., and hydrogen chloride gas is bubbled into the solution, with cooling for about 15 minutes. The solution is then stirred at room temperature for about 4 hours. The solution is evaporated in vacuo, and azeotroped with methanol, then chloroform, to give the amine product as the hydrochloride salt. The hydrochloride salt is partitioned between chloroform and sodium bicarbonate solution, and the organic layer washed with brine, dried, filtered and one equivalent of benzoic acid is added. The solvent is removed in vacuo and the residue triturated in ether to give the desired amine (ii) depicted above as the benzoate salt, m.p. 183–184° C.

Step (2) Preparation of

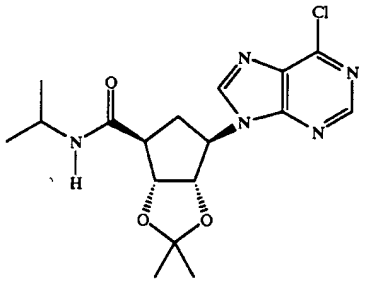
(iii)



54 mmol of the product (ii) from Example 2 Step (1) above is dissolved in 110 mL n-butanol and 9.7 g 5-amino-4,6-dichloropyrimidine, then 23 mL triethylamine were added and the mixture heated at reflux for about 18 hours. The mixture is cooled, diluted with chloroform and saturated ammonium chloride solution. The aqueous layer is extracted three times chloroform, then twice with 10% isopropyl alcohol/chloroform. The organic layers are combined, dried over sodium sulfate, filtered, concentrated to an oil (iii) which is used, without further treatment for the next step.

Step (3) Preparation of

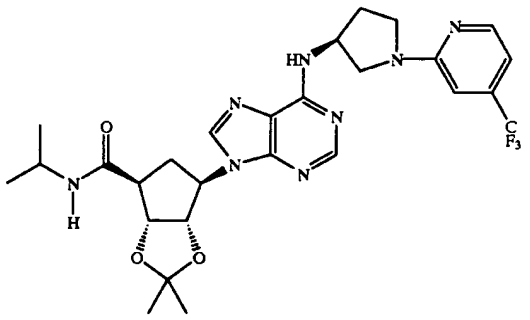
(iv)



The product (iii) from Example 2 Step (2) above is taken up in 150 mL n-butyl acetate and 11.2 g formamidine acetate is added. The mixture is heated at reflux under argon for about 9 hours, adding three 5.56 g portions of formamidine acetate at two, four, and six hours. The mixture is cooled, diluted with ethyl acetate, washed with brine, water, brine, dried over sodium sulfate, filtered, concentrated in vacuo, and the residue purified by flash chromatography, eluting with 40–80% ethyl acetate in hexane, to give the desired chloropurine product (iv) depicted above.

Step (4) Preparation of

(v)



400 mg (1.05 mmol) of the product (iv) from Example 2 Step (3) above, 0.22 mL (1.57 mmol) triethylamine, and 270 mg (1.16 mmol) 2-[(3S)-3-aminopyrrolidin-1-yl]-4-trifluoromethylpyridine (prepared as in Example 3, Steps 1 to 5, below) were dissolved together in 3 mL ethanol, and the solution heated at reflux, under argon, for about 20 hours. The mixture is evaporated in vacuo and the residue partitioned between chloroform and saturated sodium bicarbonate solution. The aqueous layer is extracted with 4 portions of chloroform and the combined organic dried over sodium sulfate, filtered, evaporated in vacuo. The residue is purified by flash chromatography, applying the sample in methylene chloride/ethyl acetate (1:1), and eluting with 0 to 3% methanol in ethyl acetate, to give the above-depicted product (v).

Step (5)The product from Example 2 Step (4) above is dissolved in 2 mL methanol/tetrahydrofuran (1:1), and 3.3 mL 1.5N aqueous hydrochloric acid is added, and the solution stirred at room temperature for about 20 hours. The

mixture is evaporated in vacuo. This resulting residue is taken up in 10 mL 15% isopropyl alcohol/chloroform, 1 mL 1N sodium hydroxide solution, and 9 mL saturated sodium bicarbonate solution. The layers are separated and the aqueous extracted with 4×5 mL portions of 15% isopropyl alcohol/chloroform. The combined organic layer is dried over sodium sulfate, filtered, evaporated in vacuo to give (1S,2R,3S,4R)-2,3-dihydroxy-4-[6-[1-(4-trifluormethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]purin-9-yl]cyclopentanecarboxylic acid isopropylamide, m.p. 227–228° C.

EXAMPLE 3

Preparation of (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol

Step (1) 20 g (232 mmol) of (3S)-(-3aminopyrrolidine and 26 mL (255 mmol, 1.1 eq) benzaldehyde are combined in 250 mL toluene and refluxed, removing water with a Dean-Stark trap, for about 4.5 hours. The mixture is cooled to 0° C. and 55.7 g (255.2 mmol, 1.1 eq) di-tert-butyl dicarbonate added, then stirred at room temperature. The mixture is concentrated in vacuo, stirred with $KHSO_4$ solution, extracted 3 times with ether. The aqueous layer is made alkaline and extracted with CH_2Cl_2. The organic layer is washed with brine and dried over $MgSO_4$, filtered, and evaporated in vacuo to give N1-BOC-(3S)-(-)-3 aminopyrrolidine.

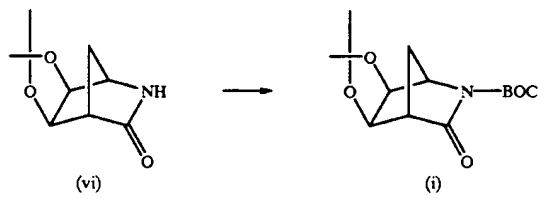
Step (2)34.25 g (183.9 mmol) of the product from Example 3 Step (1) above is dissolved in 200 mL CH_2Cl_2 and 25 mL (183.9 mmol) of triethylamine is added. Under a nitrogen atmosphere, 34.7 mL (367.8 mmol, 2 eq) of acetic anhydride is added dropwise, the mixture stirred at room temperature, partioned with $NaHCO_3$ solution/CH_2Cl_2. The organic layer is washed with brine, dried over $MgSO_4$, filtered, evaporated in vacuo, and the product purified by flash chromatography, eluting with 2–8% methanol in methylene, to give N1-BOC-(3S)-(-)-3-acetylaminopyrrolidine.

Step (3)39.2 g (171.7 mmol) of the product from Example 3 Step (2) above is dissolved in 400 mL CH_2Cl_2 and 26.46 mL (343.4 mmol 2 eq) trifluoroacetic acid (hereinafter "TFA") is dropwise at at 0° C. under a nitrogen atmosphere. The mixture is heated to reflux, adding another 26 mL, then another 10 mL of TFA, refluxed for about an additional 3 hours, then evaporated under high vacuum to remove TFA. The residue was stirred with Amberlite IRA-400 basic resin (hereinafter "basic resin"), filtered, the filtrate dissolved in methanol, filtered slowly through basic resin, and the filtrate evaporated to give (3S)-(-)-3-acetylaminopyrrolidine.

Step (4)4 g (31.2 mmol) of the product from Example 3 Step (3) above and 5.19 (40.6 mmol) 2-chloro-5-trifluoromethylpyridine are combined in 50 mL ethanol and 13 mL (93.6 mmol, 3 eq) triethylamine are added. The mixture is refluxed for about 18 hours, concentrated in vacuo and the residue partitioned between methylene chloride and sodium bicarbonate solution. The organic layer is washed with brine, dried over magnesium sulfate, filtered, evaporated in vacuo, and the residue purified by flash chromatography, eluting with 2–5% methanol in methylene chloride, to give 2-[(3S)-3-acetylaminopyrrolidin-1-yl]-5-trifluoromethylpyridine, as a solid.

Step (5)7.52 g (27.5 mmol) of the product from Example 3 Step (4) above is combined with 75 mL 6N aqueous hydrochloride acid and the mixture refluxed for about 18 hours. The mixture is cooled to room temperature, neutralized with solid sodium bicarbonate, partitioned between dilute sodium hydroxide solution and methylene chloride. The organic layer is washed with brine, dried over magnesium sulfate, filtered, evaporated in vacuo to give 2-[(3S)-3-aminopyrrolidin-1-yl]-5-trifluoromethylpyridine.

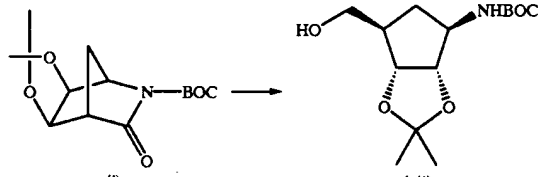
Step (6)



(vi) (i)

22.5g (0.123 mol) (-)-5,6-Dimethylenedioxy-2-azabicyclo[2.2.1]heptan-3-one (vi), 1.5 g 4-dimethylaminopyridine (hereinafter "DMAP"), 12.4 g triethylamine, and 37.5 g di-tert-butyl dicarbonate are combined in methylene chloride and stirred at room temperature for about 18 hours. The mixture is washed with 1N hydrochloric acid, 5% sodium bicarbonate solution, brine, dried over sodium sulfate, filtered, concentrated in vacuo and the residue recrystallized from isopropyl alcohol to give N-BOC-5,6-Dimethylenedioxy-2-azabicyclo[2.2.1]heptan-3-one (i).

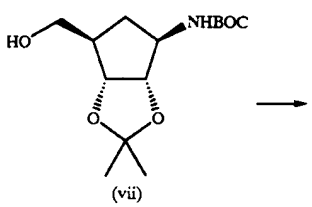
Step (7)



(i) (vii)

35.6 g (0.125 mol) of the product (i) from Example 3 Step (6) above is combined with 400 mL methanol. With rapid stirring and cooling, under argon purge, a total of 23.8 g (0.63 mol) sodium borohydride is added in three equal portions over a period of about 2 hours. The mixture is concentrated in vacuo and partitioned between 200 mL water and 300 mL ethyl acetate. The aqueous layer is extracted twice more with ethyl acetate and the combined organic solution washed with water, brine, dried over sodium sulfate, filtered, concentrated in vacuo to give N-BOC-1-amino-2,3-dimethylenedioxy-4-hydroxymethylcyclopentane (vii).

Step (8)



(vii)

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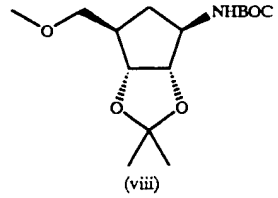
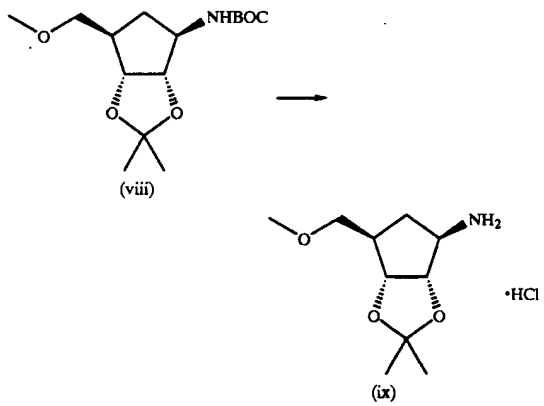


(viii)

50 g of the product (vii) from Example 3 Step (7) above is placed in 150 mL benzene. 8.8 ml methyl iodide and 33 g silver oxide are added and the mixture refluxed for about 18 hours. Another 25 g of silver oxide and another 50 mL of methyl iodide are added portionwise over about 6 hours and the mixture refluxed for about 18 hours. The mixture is filtered through Celite and the filter cake washed with ethyl acetate. The combined filtrate is concentrated in vacuo and the residue crystallized from hexane to give the desired methoxymethyl compound (viii) depicted above.

Step (9)

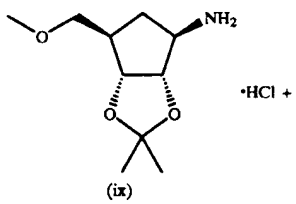


(viii)

(ix)

Under argon, 31.6 g of the product (viii) from Example 3 Step (8) above is dissolved in 250 mL warm anhydrous ethyl acetate. The solution is cooled in an ice bath and hydrogen chloride gas is bubbled through the solution for about 6 minutes. The mixture is allowed to warm to room temperature and stirred for about 3 hours, then concentrated in vacuo to give the desired amine hydrochloride (ix) depicted above.

Step (10)



(ix)

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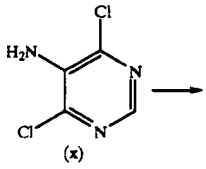
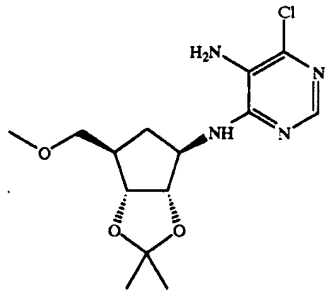


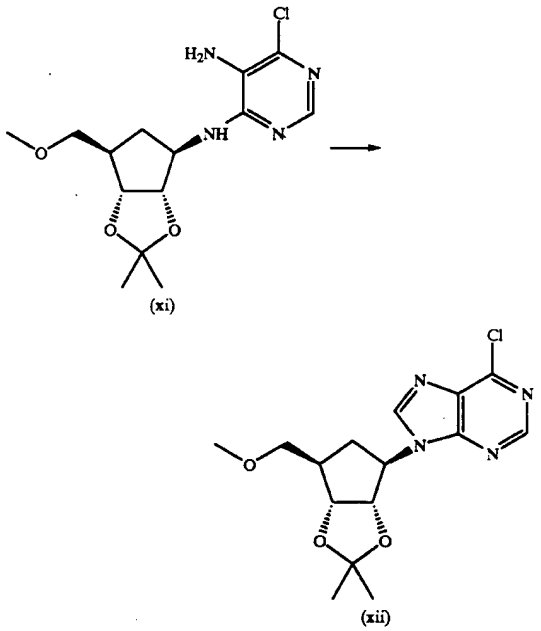
(x)



(xi)

24.2 g of the product from (ix) Example 3 Step (9) above and 42.8 g sodium bicarbonate are combined in 100 mL n-butanol, under argon, and 20.1 g 5-amino-4,6-dichloro pyrimidine is added. The mixture is heated at reflux for about 20 hours, then concentrated in vacuo. The residue is partioned between ethyl acetate and water and the ethyl acetate layer washed with brine, dried over magnesium sulfate, filtered, concentrated in vacuo. The residue in 30% ethyl acetatate in hexane, passed through a large flash silica gel wash column, and the column is washed with 50% ethyl acetate/hexane and the combined filtrates concentrated in vacuo to give the desired pyrimidinylaminocyclopentane product (xi) depicted above.

Step (11)



(xi)

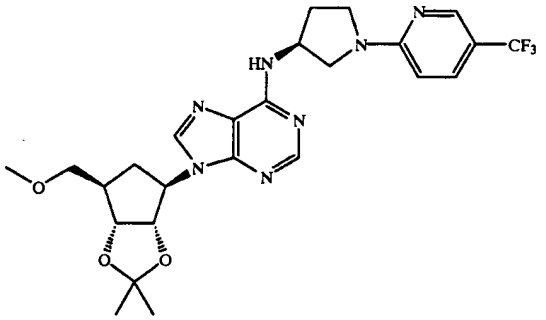
(xii)

26.7 g of the product (xi) from Example 3 Step (10) above is combined with 125 mL n-butyl acetate under argon. 33.5

g formamidine acetate added and mixture heated at reflux for about 3 hours, until thin layer chromatography shows reaction is complete. The mixture is cooled, partitioned between ethyl acetate and brine and the ethyl acetate layer dried over magnesium sulfate, filtered, concentrated in vacuo. The residue was purified by flash chromatography, eluting with 30–50% ethyl acetate in hexane, to give the chloropurine product (xii) depcited above.

Step (12) Preparation of

(xiii)



7.75 g (22.9 mmol) of the product (xii) from Example 3 Step (11) above and 6.35 g (27.4 mmol) 2-[(3S)-3-aminopyrrolidin-1-yl]-5-trifluoromethylpyridine are combined in 20 mL ethanol and 6.33 mL triethylamine added. The mixture is heated in a sealed vessel at 105° C. for about 4 hours. The mixture is cooled, evaporated in vacuo, partitioned between methylene chloride and sodium bicarbonate solution. The organic layer is dried over magnesium sulfate, filtered, concentrated in vacuo, and the residue purified by flash chromatography, eluting with 4% methanol in methylene chloride, to give the product (xiii) indicated.

Step (13) 10.81 g (20.3 mmol) of the product (xiii) from Example 3 Step (12) above is combined with 90 mL trifluoroacetate and 10 mL water, and the mixture stired at room temperature for about 30 minutes. The TFA is evaporated off at high vacuum and the residue partitioned between methylene chloride and sodium bicarbonate solution. The methylene chloride solution is washed with sodium bicarbonate solution, brine, isopropyl alcohol is added and the solution dried over magnesium sulfate, filtered, concentrated in vacuo, and the residue flash chromatographed, eluting with 5–10% methanol in methylene chloride. The appropriate fractions are collected, concentrated, and the residue crystallized from acetonitrile to give (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 166–168° C.

EXAMPLE 4

Preparation of (2R,3S,4R,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3 (R)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol

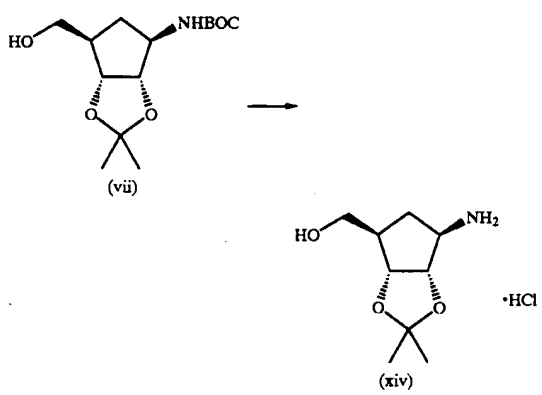
267 mg 2-[(3R)-3-aminopyrrolidin-1-yl]-5-trifluoromethylpyridine, 331 mg 6-chloropurineriboside, 233 mg triethylamine, and 0.5 mL ethanol are combined and heated in a sealed vessel at 100° C. for about 5 hours. The mixture is cooled, partioned between methylene chloride (with some isopropyl alcohol added) and sodium bicarbonate. The organic layer is washed with brine, dried over magnesium sulfate, evaporated, and the residue purified by flash chromatography, eluting with 5% methanol in methylene chloride, to give (2R,3S,4R,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(R)-ylamino]purin-9-yl]tetrahydrofuran-3,4-diol, as the hemihydrate, m.p. 166–170° C.

EXAMPLE 5

Preparation of (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl)-3-hydroxymethylcyclopentane-1,2-diol

Step (1) 1.00 g (11.6 mmol) 3(S)-(-)-3-aminopyrrolidine, 1.35 mL (9.66 mmol) 4-bromobenzotrifluoride, 2.69 g (29 mmol) sodium tert-butoxide, and 1.01 g (1.16 mmol) $PdCl_2(P[o\text{-}tolyl]_3)_2$ (prepared as in U.S. Pat. No. 4,196,135, incorporated herein by reference) are combined in 30 mL toluene, and the mixture heated in a sealed vessel at 100° C. for about 40 hours. The mixture was cooled, filtered, evaporated in vacuo and the residue purified by flash chromatography, eluting with 10:1 to 7:1 methylene chloride/ethanol, to give 1-(4-trifluoromethyl)phenyl-(3S)-pyrrolidin-3-ylamine.

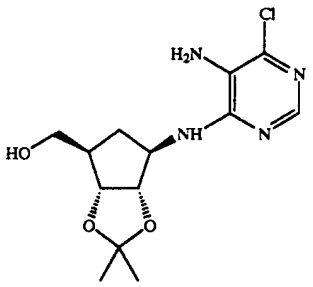
Step (2)



A solution of 24.7 mL (0.61 mol) methanol and 50 mL ethyl acetate is cooled to 0° C., under argon. 43.3 mL (0.61 mol) acetyl chloride is added portionwise and the solution allowed to come to room temperature over about 45 minutes. This solution is again cooled in ice and a solution of 50.0 g N-BOC-1-amino-2,3-dimethylenedioxy-4-hydroxymethyl cyclopentane (vii) in 100 mL ethyl acetate is added over a period of about 45 minutes. The solution is allowed to come to room temperature, then evaporated in vacuo to give the desired amine hydrochloride (xiv) depicted above.

Step (3) Preparation of

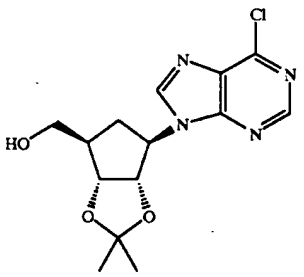
(xv)



38.9 g of the product (xiv) from Example 5 Step (2) above and 73 g sodium bicarbonate are combined in 150 mL n-butanol under argon, and the mixture stirred at room temperature for about 30 minutes. 34.2 g 5-amino-4,6-dichloropyrimidine is added and the mixture stirred at reflux for about 19 hours. The mixture is concentrated in vacuo, and the residue taken up in ethyl acetate and water. The aqueous layer is extracted with ethyl acetate and the combined organic washed with brine, filtered, concentrated in vacuo. The residue is purified by flash chromatography, eluting with a gradient of 30% to 100% ethyl acetate in hexane, to give the desired substituted chloropyrimidine (xv) depicted above.

Step (4) Preparation of

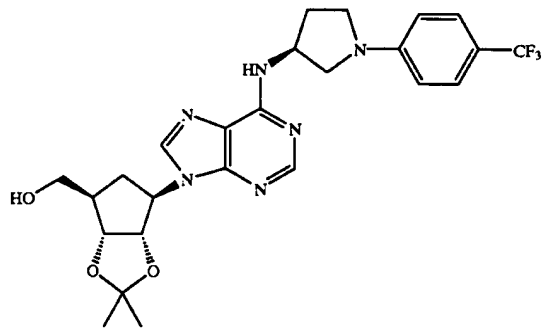
(xvi)



37.9 g of the product (xv) from Example 5 Step (3) above and 25.1 g formamidine acetate are combined in 250 mL n-butyl acetate and the mixture heated at reflux, under argon, for about 2 hours, adding an additional 12.5 g formamidine acetate after about 1 hour, and an additional 10 g after about 1.5 hours. The mixture is cooled, partitioned between ethyl acetate and brine, the brine extracted with 3 portions of ethyl acetate, and the combined organic dried over magnesium sulfate, filtered, evaporated in vacuo. The residue is purified by crystallization from ethyl acetate/hexane to give the above-depicted chloropurine (xvi). The residue from concentration of the mother liquor can be purified by flash chromatography, eluting with 80 to 100% ethyl acetate in hexane to improve recovery.

Step (5) Preparation of

(xvii)



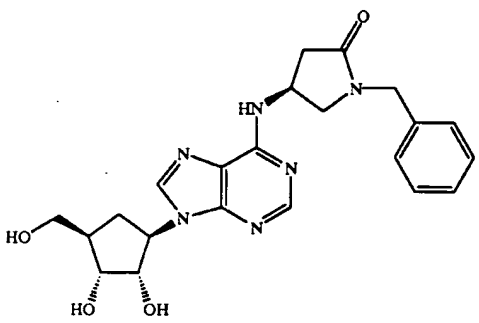
0.225 g (0.693 mmol) of the product (xvi) from Example 5 Step (4) above, 0.239 g (1.04 mmol) 1-(4-trifluoromethyl) phenyl-(3S)-pyrrolidin-3-ylamine, from Step (1) above, and 0.582 g (6.93 mmol) sodium bicarbonate are combined in 20 mL ethanol and heated at reflux for about 60 hours. The mixture is filtered, concentrated in vacuo, and the residue purified by flash chromatography, eluting with a gradient of methylene chloride/ethanol, 30:1 to 10:1, to give the pyrrolidinylamine (xvii) depicted above.

Step (6)0.234 g of the product from Example 5 Step (5) above is dissolved in 10 mL trifluoroacetic acid and the solution stirred at room temperature overnight. The solution is evaporated in vacuo, and the residue purified by flash chromatography, eluting with methylene chloride/ethyl acetate (10:1) to give (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 111–114° C.

EXAMPLE 6

Preparation of 4(S)-1-benzyl-4-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino] pyrrolidin-2-one

(xviii)



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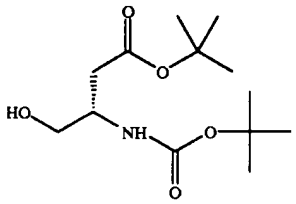
Step (1) Preparation of

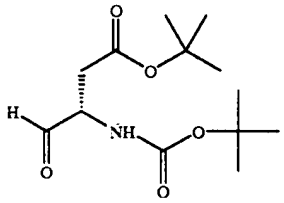
(xix)



7.1 g (24.5 mmol) N-t-BOC-L- aspartic acid β-t-butyl ester is dissolved in 120 mL tetrahydrofuran. The solution is cooled to 0° C. and 2.73 g (27 mmol) triethylamine, then 2.66 g (24.5 mmol) ethyl chloroformate is added. The solution is stirred for about 30 minutes, and a solution of 3.71 g (98.2 mmol) sodium borohydride in water is added. The mixture is stirred at room temperature for about 17 hours, concentrated in vacuo and the residue diluted with ethyl acetate, and the organic layer washed with 1N hydrochloric acid, 10% sodium carbonate, brine, then dried over magnesium sulfate, filtered, concentrated in vacuo and the residue purified by flash chromatography, eluting with 30% to 50% ethyl acetate in hexane, to give 3(S)-t-butyl-3-BOC-amino-4-hydroxy-n-butanoate (xix).

Step (2) Preparation of

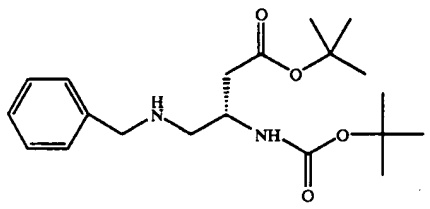
(xx)



A solution of 0.73 g of dimethylsulfoxide in 9 mL of methylene chloride is cooled to −70° C. and 31 mL of a 2M solution of oxalyl chloride in methylene chloride is added dropwise. The solution is stirred for about 15 minutes and a solution of 0.85 g of 3(S)-t-butyl-3-BOC-amino-4-hydroxy-n-butanoate (xix) in 5 mL methylene chloride is added. After stirring for about 45 minutes, 1.88 g triethylamine is added. The solution is allowed to warm to room temperature, stirred for about 30 minutes, then diluted with ethyl acetate. The solution is washed with 1N hydrochloric acid, 10% sodium carbonate, brine, dried over magnesium sulfate, filtered, concentrated in vacuo, to give give 3(S)-t-butyl-3-BOC-amino-4-oxo-n-butanoate (xx).

Step (3)

(xxi)



The product (xx) from Example 6 Step (2) above is dissolved in 9 mL methanol and 1.34 g benzyl amine hydrochloride, then 0.94 g triethylamine, then 200 mg 3 Å molecular seives. The solution is stirred for about 45 minutes and a solution of 0.23 g zinc chloride and 0.22 g sodium cyanoborohydride in 5 mL methanol is added. The solution is stirred for about 4 hours, 2 mL 1N sodium hydroxide, then 10 mL water are added, the mixture concentrated to about one-half volume, and extracted with ethyl acetate. The ethyl acetate solution is washed with 10% sodium carbonate solution, brine, dried over magnesium sulfate, filtered, concentrated in vacuo, and the residue purified by flash chromatography, eluting with 30% to 40% ethyl acetate in hexane, to give the benzyl amine (xxi) depicted above.

Step (4)0.90 g of the product from Example 6 Step (3) above is dissolved in 12 mL of toluene/acetic acid (10:1), and the solution refluxed for about 1.5 hours. The mixture is concentrated in vacuo, and the residue purified by flash chromatography, eluting with 25%–35% ethyl acetate in methylene chloride, to give 1-benzyl-4(S)-BOC-amino-2-pyrrolidinone.

Step (5)0.64 g of the product from Example 6 Step (4) above is dissolved in 20 mL ethyl acetate and the solution cooled to 0° C. Hydrogen chloride gas is bubbled into the solution for about 5 minutes, and the mixture stirred at room temperature for about 18 hours. Ether is added to the mixture and the solid collected by filtration to give 1-benzyl-4(S)-amino-2-pyrrolidinone hydrochloride.

Step (6) 0.33 g of the protected chloropurine from Example 5, Step (4) above, 0.26 g 1-benzyl-4(S)-amino-2-pyrrolidinone hydrochloride, and 0.29 g triethylamine are combined in 10 mL ethanol and the mixture heated at reflux for about 50 hours. The mixture is concentrated in vacuo and the residue dissolved in 20 mL 1N hydrochloric acid and stirred at room temperature for about 1 hour. The mixture is concentrated in vacuo and the residue purified by preparative HPLC, eluting with a gradient of 10% acetonitrile to 60% acetonitrile in water, containing 0.1% trifluoroacetic acid. The appropriate fractions were combined, concentrated, and the residue dissolved in 20 mL 1N hydrochloric acid, the solvent evaporated in vacuo, and this repeated twice more. This residue was dissolved in methanol, the solvent evaporated in vacuo, and the residue triturated in ether to give 4(S)-1-benzyl-4-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6ylamino]pyrrolidin-2-one as the hydrochloride trihydrate, m.p. 100° C. (dec.)

EXAMPLE 7

Preparation of (1S,2R,3R,5R)-3-hydroxymethyl-5-[6[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentane-1,2-diol

Step (1)4-nitrophenol (1.0 g, 7.19 mmol) and triethylamine (3 mL, 21.6 mmol) were dissolved together in anhydrous methylene chloride (10 mL), and the solution cooled to −15° C. Trifluoromethanesulfonic anhydride (1.81 mL, 10.8 mmol) is added and the mixture stirred at −15° C. for about 30 minutes. The mixture is diluted with methylene chloride, washed with sodium bicarbonate solution and brine, the organic layer dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue is purified by flash chromatography, eluting with methylene chloride, to give 4-nitrophenyl trifluoromethanesulfonate as a light yellow solid.

Step (2)3(S)-amino-1-benzylpyrrolidine (3.0 g, 17.0 mmol) and triethylamine (2.50 mL, 17.9 mmol) are dissolved together in anhydrous methanol (17 mL), under

nitrogen, and ethyl trifluoroacetate (2.53 mL, 21.3 mmol) is added dropwise. The solution is stirred for about 18 hours, evaporated in vacuo, and the residue taken up in methylene chloride. The solution is washed with sodium bicarbonate solution, brine, dried over magnesium sulfate, filtered, concentrated in vacuo to give 1-benzyl-3(S)-trifluoroacetylaminopyrrolidine.

Step (3)Under nitrogen, 1-benzyl-3(S)-trifluoroacetylaminopyrrolidine (4.59 g, 16.7 mmol) is dissolved in anhydrous methanol (50 mL) and di-tert-butyl dicarbonate (3.68 g, 16.7 mmol) and 10% palladium on carbon (0.90 g) are added. The mixture is then stirred under hydrogen under atmospheric pressure for about 5 hours. The mixture is filtered through Celite®, rinsing with methanol, and the filtrate evaporated in vacuo, The residue was purified by flash chromatography, eluting with 5% methanol in methylene chloride to give 1-BOC-3(S) trifluoroacetylaminopyrrolidine.

Step (4)1-BOC-3(S)-trifluoroacetylaminopyrrolidine (4 g) is dissolved in methylene chloirde (130 mL) and trifluoroacetic acid (19 mL) is added. The solution is stirred at room temperature for about 1 hour, then concentrated in vacuo. The residue is partitioned between methylene chloride and saturated sodium bicarbonate solution. The layers are separated and the aqueous extracted with ethyl acetate. The combined organic is dried over magnesium sulfate, filtered, evaporated in vacuo to give 3(S)-trifluoroacetylaminopyrrolidine.

Step (5)4-Nitrophenyl trifluoromethanesulfonate (0.423 g, 1.56 mmol) and triethylamine (0.217 mL, 1.56 mmol) are dissolved together in anhydrous acetonitrile (15 mL) and 3(S)-trifluoroacetylaminopyrrolidine (0.852 g, 4.68 mmol) is added and the mixture heated at reflux for about 18 hours. The mixture is cooled, concentrated in vacuo and the residue purified by flash chromatography, eluting with a gradient of 25% to 50% ethyl acetate in hexane to give 1-(4-nitro) phenyl-3(S)-trifluoroacetylaminopyrrolidine.

Step (6)1-(4-Nitro)phenyl-3(S)-trifluoroacetylaminopyrrolidine (0.334 g, 1.10 mmol) is combined with a saturated solution of potassium carbonate in methanol/water (2:3) (20 mL), and the mixture heated at 55° C. for about two hours, then at room temperature for about 18 hours. The mixture is concentrated in vacuo and the residue taken up in water (10 mL). The aqueous is extracted with ethyl acetate, and the organic dried over magnesium sulfate, filtered, evaporated in vacuo to give 3(S)-amino-1-(4-nitro)phenylpyrrolidine.

Step (7)Using essentially the procedures of Example 3, Steps 12 and 13, and Example 5, Steps 5 and 6, (1S,2R,3R, 5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 119–120° C., is prepared from 3(S)-amino-1-(4-nitro) phenylpyrrolidine.

Using essentially the procedures of the Reaction Schemes and Examples as described hereinabove, the following compounds are prepared from the appropriate starting materials:

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-chloropyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, m.p. 154–156° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 153–156° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 187–190° C.;

(2R,3R,4S,5R) 2-hydroxymethyl-5-[6-[1-(5-bromopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, 153–154° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-(6-(1-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino)-purin-9-yl) tetrahydrofuran-3,4-diol, m.p. 230–232° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(5'-trifluoromethyl-3,4,5,6-tetrahydro-2H-[1,2']-bipyridinyl-3-yl)-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 113–116° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(phenylpyrrolidin-3(S)-ylamino)-purin-9-yl]tetrahydrofuran-3,4-diol;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(1-pyridin-2-ylpyrrolidin-3(S)-ylamino]-purin-9-yl] tetrahydrofuran-3,4-diol, m.p. 193–195° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, m.p. 121–124° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 164–166° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-thiophen-2-ylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl] tetrahydrofuran-3,4-diol, 190–192° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylmercaptopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 231–233° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 251–253° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, 154–156° C.;

(2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, m.p. 130° C. (dec.);

(2R,3R,4S,5R)-2-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, m.p. 198–200° C.;

(1S,2R,3S,4R)-2,3-dihydroxy-4-[6-[1-(5-trifluormethylpyridin-2-yl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentanecarboxylic acid ethylamide, m.p. 135–138° C.;

(1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl) piperidin-4-yl]-purin-9-yl]cyclopentane-1,2-diol, m.p. 126–128° C.;

(1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3S)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol dihydrochloride, m.p. 160° C. (dec);

(1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(R)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 175–177° C.;

(1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3R)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol, m.p. 166° C. (dec);

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 110–111 ° C.;

4(R)-1-benzyl-4-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino] pyrrolidin-2-one hydrochloride, m.p. 110° C. (dec);

(1R,2S,3R,5S)-5-methyl-3-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 114–116° C.;

(1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 169–171° C.;

(1R,2S,3R,5R)-5-[6-[1-(5-chloropyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 118–121 ° C.;

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 135–137° C.;

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(pyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 110–112° C.;

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(quinolin-3-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 135–138° C.;

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-S-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol;

(1R,2S,3R,5R)-5-[6-[1-(4,5-bistrifluorpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 123–126° C.;

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(phenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 97–99° C.;

4-[3(S)-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino] pyrrolidin-1-yl]benzonitrile, m.p. 140° C.;

(1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(isoquinolin-1-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 119–122° C.;

(1R,2S,3R,5R)-5-[6-[1-(6-bromoquinolin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol;

(1R,2S,3R,5R)-5-[6-[1-(4-chlorophenyl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol;

(1R,2S,3R,5R)-3-[6-[1-(3-chloro-5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, m.p. 140–143° C.;

(1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 180–182° C.;

(1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 125–127° C.;

(1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol;

(1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol;

(1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 118–120° C.;

(1R,2S,3R,5R)-3-isopropoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 157–158° C.;

(1R,2S,3R,5R)-3--isopropoxymethyl-5-[6-[1-(4-trifluoromethylpyridin2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 160–161 ° C.;

(1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, m.p. 122–124° C.;

(1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, m.p. 110–111° C.;

(1R,2S,3R,5R)-5-[6-[1-(5-chlorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, m.p. 110–112° C.;

(1R,2S,3R,5R)-3-methoxymethyl-5-[6[1-(4-trifluoromethylphenyl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, m.p. 128° C.;

(1R,2S,3R,5R)-5-(-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, m.p. 122–125° C.;

(1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, m.p. 127–130° C.;

(1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, m.p. 131–133° C.; and

(1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-phenylpyrrolidin-3-(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol, m.p. 106° C.

(1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino) purin-9-yl]5-hydroxymethylcyclopentane-1,2-diol, m.p. 100–102° C.;

(1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino) purin-9-yl]5-methoxymethylcyclopentane-1,2-diol, m.p. 95–96° C.

5'-N-[1(S)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl] carbocyclic adenosine-5'-uronamide, m.p. 215° C. (dec.); and

5'-N-[1(R)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl] carbocyclic adenosine-5'-uronamide, m.p. 206–212° C. (dec.).

Compounds of the present invention are useful as antihypertensive agents for the treatment of high blood pressure; they also increase coronary blood flow, and, accordingly, are useful in the treatment of myocardial ischemia; they also act as cardioprotective agents useful for the prevention or reduction of injury to the myocardium consequent to myocardial ischemia; and they also act as antilipolytic agents useful for the treatment of hyperlipidemia and hypercholesterolemia.

Compounds within the scope of this invention exhibit activity in standard A_1/A_2 receptor binding assays for the determination of adenosine receptor agonist activity in mammals. Exemplary test procedures which are useful in determining the receptor binding affinity of compounds of the present invention are described below.

A. IN VITRO ADENOSINE RECEPTOR BINDING AFFINITY DETERMINATION

A_1 Receptor Binding Affinity was determined by competition assay based on ligand displacement of ^{3}H-CHA (cyclohexyl adenosine) [Research Biochemicals Inc., Natick, Mass.] from receptor using a membrane preparation of whole rat brain, according to the procedure of R. F. Bruns et al., Mol. Pharmacol., 29:331 (1986). Non-specific binding was assessed in the presence of 1 mM theophylline.

A_2 receptor binding affinity was determined by a similar assay technique, based on ligand displacement of ^{3}H-CGS

21680, a known A_2 receptor-specific adenosine agonist, from receptor, using membranes from rat brain striatum. Non-specific binding was assessed in the presence of 20 μM 2-chloroadenosine.

The assays were run in glass test tubes in duplicate at 25_C. Once the membranes were added, the tubes were vortexed and incubated at 25_C. for 60 minutes (A_1 assay) or 90 minutes (A_2 assay) on a rotary shaker. The assay tubes were vortexed halfway through the incubation and again near the end. The assays were terminated by rapid filtration through 2.4 cm GF/B filters using a Brandel Cell Harvestor. The test tubes were washed three times with cold 50 mM tris-HCl (pH 7.7 or 7.4), with filtration being completed within 15 seconds. The damp filter circles were placed in glass scintillation vials filled with 10 mL of Aquasol II (New England Nuclear). The vials were allowed to shake overnight on a rotary shaker and were placed into a liquid scintillation analyzer for two minute counts. IC_{50} values for receptor binding, i.e. the concentration at which a compound of the invention displaced the radiolabeled standard, were obtained using a curve-fitting computer program (RS/1, Bolt, Beranek and Newman, Boston, Mass.).

A_1 Receptor Binding Affinity was determined also using a preparation of rat epididymal fat pad membranes.

Membrane Preparation: Rat epididymal fat pads are homogenized in buffer containing 0.25M Sucrose, 10 mM Tris, 2 mM EDTA, 0.1M phenylmethylsulfonylfluoride, and 1 μg/mL Leupeptin (200 mg wet tissue weight/mL buffer). This homogenate is placed into 50 mL centrifuge tubes and centrifuged at 1000 g (3000 RPM) for 1 minute, the intermediate supernatent is removed and centrifuged at 38,000 g for 15 minutes. The pellets are resuspended pellets in assay buffer (50 mM Tris and 1 mM EDTA) (300 mg original tissue weight/mL assay buffer), and 2 μl/ml of a solution of adenosine deaminase (10 mg/ml) is added to the suspension and the suspension incubated for 30 minutes at 37° C. The suspension is centrifuged at 38,000 g for 10 minutes, the pellet washed once with 20 ml assay buffer, the resuspended in assay buffer (1.2 g original wet tissue weight/mL buffer).

Assay and Counting: Tubes are prepared as follows: Totals (total counts bound) tubes, 100 μL membrane suspension (prepared as described above), 50 μL ^{3}H-cyclohexyladenosine solution (prepared by diluting a solution of approximately 1 mCi/mL, with a specific activity of approximately 29.9 Ci/mmol, with assay buffer to 100 nM, hereinafter "CHA solution"), 350 μL assay buffer; Non-specific binding tubes, 100 μL membrane suspension, 50 μL CHA solution, 50 μL 100 μM 2-chloroadenosine in assay buffer, 300 μL assay buffer; Sample tubes, 100 μL membrane suspension, 50 μL CHA solution, 50 μL of a solution of the compound to be tested (which may be prepared from serial dilution in assay buffer of a DMSO solution), 300 μL assay buffer; Blank tubes, 50 μL CHA solution, 450 μL assay buffer. Each tube is vortexed for 10 seconds, incubated at 23° C. for two hours, and filtered using a Brandel Filtration Unit, using Whatman GF/B Filter Paper, washing twice with 5 mL 50 mM Tris. The filter discs are placed in 7 mL scintillation vials, which are then filled with approximately 5 mL Ready Safe Scintillation Cocktail, and counted.

B. IN VITRO VASORELAXATION DETERMINATION IN ISOLATED SWINE CORONARY ARTERIES

Swine coronary arteries were obtained from a local slaughter house, dissected carefully and cleaned of fat, blood and adhering tissue. Rings approximately 2–3 mm wide were cut and transferred to water-jacketed tissue baths (10 mL) filled with warm (37_C.), oxygenated (O_2/CO_2:95%/5%) Krebs-Henseleit buffer and mounted on L-shaped books between stainless steel rods and a force transducer. The composition of the Krebs buffer is as follows (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$,1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 10.0. Rings were equilibrated for 90 minutes with frequent buffer changes at a resting tension of 5 g In order to assure optimal tension development, arterial rings were primed twice with 36 mM KCl and once with 10 μm. PGF2a, before being exposed to 3 μM PGF2a . When isometric tension had reached a steady state, accumulative doses of the adenosine analogues of the invention (usually 1 mM to 100 μM, in half logs) were added to the baths. Tension achieved with 3 μM PGF2a was considered equivalent to 100%; all other values were expressed as a percentage of that maximum. IC_{50} values for relaxation, i.e. the concentration at which a compound of the invention caused a 50% reduction in tension, were determined using the above-mentioned linear curve fitting computer program.

C. IN VIVO MEAN ARTERIAL BLOOD PRESSURE (MAP) AND HEART RATE (HR) DETERMINATIONS IN NORMOTENSIVE ANESTHETIZED AND SPONTANEOUSLY HYPERTENSIVE RAT

1. Anesthetized Rat

Normotensive rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed on a heated surgical table. Cannulas were inserted into the femoral artery and veined to allow the measurement of arterial pressure and to facilitate the intravenous administration of test compounds. The animals was allowed to equilibrate for 10 minutes after surgery. Mean arterial pressure was continuously measured and recorded and heart rate was monitored using the arterial pressure pulse to trigger a cardiotachometer. After baseline parameters were established and recorded, increasing doses (1, 3, 10, 30, 100, 300 and 1000 μg/kg) of the compound of the invention to be tested were administered intravenously. Maximal changes in the cardiovascular parameters were observed after each dose of the adenosine analogue. Only one compound was administered per rat. The potency of the compounds to lower heart rate and mean arterial pressure were assessed by determining the dose of the agent necessary to lower the heart rate or arterial pressure by 25% (ED_{25}).

2. Spontaneously Hypertensive Rat (SHR)

The oral antihypertensive activity of compounds of the invention were examined in conscious spontaneously hypertensive rats. The rats were anesthetized with sodium pentabarbatol (50 mg/kg i.p.). A telemetry transducer was implanted into the rats abdomen via midline incision. The cannula of the transducer was inserted into the abdomenal aorta to allow direct measurement of arterial pressure in the conscious SHR. The transducer was secured to the abdomenal wall. After recovery from surgery (minimum of seven days), the SHR were placed on a receiver plate and the transducer/transmitter was activated. Systolic, diastolic and mean arterial pressure and heart rate were recorded for 1.5 hours in the unrestrained conscious rat to establish a stable baseline. Each rat then received a single dose of the compound of the invention to be tested, or vehicle, and changes in arterial pressure and heart rate were monitored for 20 hours and recorded.

When the blood flow to the heart is interrupted for brief periods of time (2 to 5 minutes), followed by restoration of blood flow (reperfusion), the heart becomes protected against the development of injury when the blood flow is interrupted for longer periods of time (for example, 30 minutes).

D. IN VITRO HEART RATE DETERMINATION

Rat isolated atria

Male Sprague-Dawley rats are anesthetized using Ketamine/Rompun and the hearts are excised quickly and placed into warm, oxygenated (95 O_2/5% CO_2) Krebs Henseleit buffer of the following composition [mM]: NaCl 118; KCl 4.7; $CaCl_2$2.5; $MgSO_4$1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0 and glucose 10.0 (pH 7.4). The right, spontaneously beating, atria are dissected and suspended in water-jacketed tissue baths using stainless steel wires. Atria are equilibrated for 60 min at a resting tension of 2 g with buffer changes every 5 min for the first 15 min, then at 15 min intervals. Compounds of the present invention to be tested are added cumulatively to the baths and heart rate is determined using a Grasse® Model 7D polygraph.

Compounds of the invention exhibit activity in tests used to determine the ability of compounds to mimic the cardioprotective activity of myocardial preconditioning. Exemplary test procedures which are useful in determining the cardioprotective activity of compounds of the present invention are described below.

E. DETERMINATION OF CARDIOPROTECTIVE ACTIVITY IN RAT

1. General Surgical Preparation

Adult Sprague-Dawley rats are anesthetized with Inactin (100 mg/kg i.p.). The trachea is intubated and positive pressure ventilation is provided via a small animal respirator. Catheters are placed in the femoral vein and artery for administration of compounds of the present invention to be tested, and measurement of blood pressure, respectively. An incision is made on the left side of the thorax over the pectoral muscles, and the muscles are retracted to expose the fourth intercostal space. The chest cavity is opened and the heart is exposed. A length of 4-0 proline suture is placed through the ventricular wall near the left main coronary artery and is used to interrupt blood flow through the cornary artery by tightening a slip-knot. A pulsed-Doppler flow probe (a device which measures blood flow) is placed on the surface of the heart to confirm that the coronary artery has been porperly identified. A catheter is also placed in the left ventricle to monitor left ventricular function during the experiment.

2. Preconditioning and Test Procedures

For preconditioning the heart, the coronary artery is occluded (flow is interrupted) for a period of two minutes. The slip-knot is then released to restore flow (reperfusion) for a period of three minutes. This procedure of occlusion/reperfusion is repeated twice. Five minutes after completion of the final preconditioning event, the artery is reoccluded for 30 minutes, followed by reperfusion for three hours. When a compound of the present invention is being tested, instead of performing the occlusion/reperfusion procedure, the compound is infused for 30 minutes prior to the 30-minute occlusion period. At the conclusion of the 3-hour reperfusion period the artery is reoccluded and 1 mL of Patent Blue dye is administered into the left ventricular catheter and the heart is stopped by i.v. administeration of potassium chloride. This procedure allows the dye to perfuse the normal areas of the heart while that portion of the heart that was made ischemic does not take up the dye (this is the area at risk, the "risk area"). The heart is quickly removed for analysis of infarct size. Infarct size is determined by slicing the heart from apex to base into four to five slices 1–2 mm thick. Slices are incubated in a solution of 1% triphenyltetrazolium for 15 minutes. This stain reacts with viable tissue and causes it to develop a brick-red color. The infarcted tissue does not react with the stain and is pale white in appearance. The tissue slices are placed in a video image analysis system and infarct size is determined by planimetry. The effect of the compound of the present invention tested on myocardial infarct size is assessed and used to quantitate the extent of cardioprotective activity. Results are given as the percentage of the risk area which is infarcted.

Compounds of the present invention exhibit activity in tests used to determine the ability of compounds to inhibit lipolysis. Exemplary test procedures which are useful in determining the antilipolytic activity of compounds of the present invention are described below.

F. DETERMINATION OF ANTILIPOLYTIC ACTIVITY IN RAT ADIPOCYTES

1. Isolation of Adipocytes from Epididymal Fat Pads

Adipose tissue is removed from anesthetized rats and rinsed twice in incubation medium (2.09 g sodium bicarbonate and 0.04 g EDTA, disodium salt, in 1 L Krebs buffer). Each rat (300–350 g) yields approximately 4 mL of adipose tissue. The adipose tissue (35 mL) is cut into small pieces with scissors and washed with incubation medium (50 mL). The mixture is poured into the barrel of a 50 mL syringe to which is attached a short piece of clamped tubing instead of a needle. The aqueous phase is allowed to drain. A second wash with incubation medium is passed through the syringe. The tissue is added to 50 mL of collagenase solution (collagenase (90 mg), bovine serum albumin (BSA) (500 mg), and 0.1M calcium chloride solution (1 mL), in incubation medium (50 mL)) in a 1 L bottle. The mixture is shaken in an environmental at 37° C. for about 60 minutes under an atmosphere of 95% oxygen/5% carbon dioxide to effect digestion of the tissue. The dispersed cells are poured through 2 layers of cheese cloth into a 100 mL plastic beaker. The undigested clumps in the cloth are rinsed once with incubation medium (20 mL). The cells in the beaker are centrifuged in 2 plastic tubes for 30 seconds at room temperature at 300 rpm. The aqueous phase is aspirated from beneath the loosely packed layer of floating fat cells and discarded. The adipocytes are gently poured into a 250 mL plastic beaker containing 100 mL of rinse solution (1 g BSA per 100 mL incubation medium). After gentle stirring the centrifugation step is repeated. Another wash with rinse solution follows. The cells are pooled and their volume is estimated with a graduated cylinder. The adipocytes are diluted in twice their volume of assay buffer (incubation medium (120 mL), BSA (1.2 g), pyruvic acid (13 mg)).

2. In Vitro Lipolysis Assay

The assay is performed in 20 mL plastic scintillation vials and the total assay volume is 4.2 mL. Assay buffer (2.5 mL), diluted adipocytes (1.5 mL), and a solution of the compound to be tested (12.3 μL) adenosine agonist (12.3 μl; varying concentration) is incubated in the environmental shaker for 15 minutes, then the reaction is started with norepinephrine solution (41.2 μL) (10 nM, in a carrier solution containing water (100 mL), BSA (4 mg), and 0.1M EDTA (20 μL))and adenosine deaminase (1 μg/mL, 41.2 μl). After sixty minutes

in the shaker the reaction is terminated by putting the vials on ice. The contents of each vial is transferred into a 12×75 mm glass tube and centrifuged at 8–10° C. at 3600 rpm for 20 min. The hard lipid layer is removed by aspiration and the aqueous layer is assayed for glycerol (400 μl of sample). The positive control is done in the absence of any adenosine agonist, substituting water in place of the solution to be tested.

The antilipolytic activity of adenosine is mediated through activation of the A_1 receptor subtype. Selective agonists of the A_2 receptor subtype, such as CGS 21680, do not exhibit antilipolytic activity. Accordingly, while certain A_1 selective agonists may not have desirable antihypertensive activity and A_2 agonists may not be effective antilipolytic agents, compounds of the present invention which are mixed agonists are uniquely suited to effectively treat both risk factors discussed hereinabove, i.e., hypertension and hyperlipidemia.

The compounds of this invention can be normally administered orally or parenterally, in the treatment of patients suffering from hypertension, myocardial ischemia, or in patients in need of cardioprotective therapy or antilipolytic therapy. As used herein, the term "patients" includes humans and other mammals.

The compounds of this invention, preferably in the form of a salt, may be formulated for administration in any convenient way, and the invention includes within its scope pharmaceutical compositions containing at least one compound according to the invention adapted for use in human or veterinary medicine. Such compositions may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers or excipients. Suitable carriers include diluents or fillers, sterile aqueous media and various non-toxic organic solvents. The compositions may be formulated in the form of tablets, capsules, lozenges, troches, hard candies, powders, aqueous suspensions, or solutions, injectable solutions, elixirs, syrups and the like and may contain one or more agents selected from the group including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a pharmaceutically acceptable preparation.

The particular carrier and the ratio of the adenosine analogues to carrier are determined by the solubility and chemical properties of the compounds, the particular mode of administration and standard pharmaceutical practice. For example, excipients such as lactose, sodium citrate, calcium carbonate and dicalcium phosphate and various disintegratants such as starch, alginic acid and certain complex silicates, together with lubricating agents such as magnesium stearate, sodium lauryl sulphate and talc, can be used in producing tablets. For a capsule form, lactose and high molecular weight polyethylene glycols are among the preferred pharmaceutically acceptable carriers. Where aqueous suspensions for oral use are formulated, the carrier can be emulsifying or suspending agents. Diluents such as ethanol, propylene glycol, glycerin and chloroform and their combinations can be employed as well as other materials.

For parenteral administration, solutions or suspensions of these compounds in sesame or peanut oil or aqueous propylene glycol solutions, as well as sterile aqueous solutions of the soluble pharmaceutically acceptable salts described herein can be employed. Solutions of the salts of these compounds are especially suited for administration by intramuscular and subcutaneous injection. The aqueous solutions, including those of the salts dissolved in pure distilled water, are suitable for administration by intravenous injection, provided that their pH is properly adjusted, and that they are suitably buffered, made isotonic with sufficient saline or glucose and sterilized by heating or by microfiltration.

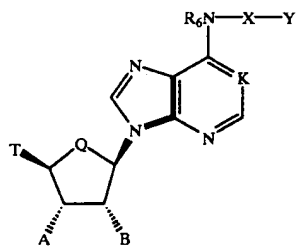
The dosage regimen used in carrying out the methods of this invention is that which insures maximum therapeutic response until improvement is obtained and thereafter the minimum effective level which gives relief. Thus, in general, the dosages are those that are therapeutically effective in lowering blood pressure in the treatment of hypertension, in increasing coronary blood flow in the treatment of myocardial ischemia, in producing a cardioprotective effect, i.e., amelioration of ischemic injury or myocardial infarct size consequent to myocardial ischemia, or in producing an antilipolytic effect. In general, the oral dose may be between about 0.1 and about 100 (preferably in the range of 1 to 10 mg/kg), and the i.v. dose about 0.01 to about 10 mg/kg (preferably in the range of 0.1 to 5 mg/kg), bearing in mind, of course, that in selecting the appropriate dosage in any specific case, consideration must be given to the patient's weight, general health, age and other factors which may influence response to the drug.

The compounds of the invention may be administered as frequently as is necessary to achieve and sustain the desired therapeutic response. Some patients may respond quickly to a relatively large or small dose and require little or no maintenance dosage. On the other hand, other patients may require sustained dosing from about 1 to about 4 times a day depending on the physiological needs of the particular patient. Usually the drug may be administered orally about 1 to about 4 times per day. It is anticipated that many patients will require no more than about one to about two doses daily.

It is also anticipated that the present invention would be useful as an injectable dosage form which may be administered in an emergency to a patient suffering from acute hypertension or myocardial ischemia, or a patient in need of cardioprotection or antilipolytic therapy. Such treatment may be followed by intravenous infusion of the active compound and the amount of compound infused into such a patient should be effective to achieve and maintain the desired therapeutic response.

What is claimed is:

1. A compound of the formula:



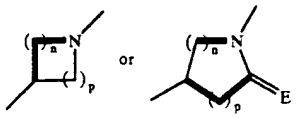
wherein:

K is N;

Q is CH_2 or O;

R_6 is hydrogen, alkyl, allyl, 2-methyl allyl, 2-butenyl, or cycloalkyl;

X is:



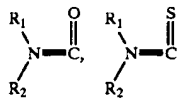
where the nitrogen of the ring of X is substituted by Y;

E is O or S;

Y is hydrogen, alkyl, aralkyl, substituted aralkyl, aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, hetereocyclylalkyl, or substituted heterocyclylalkyl, said heterocyclyl having from 4 to 10 ring members comprising one or more heteroatoms selected from the group consisting of N, O and S; and

n and p are independently with 0, 1, 2, or 3, provided that n+p is at least 1;

T is hydrogen, alkyl, alkyl carbonyl, alkyl thiocarbonal, halo, carboxyl



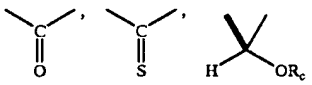
or $R_3O—CH_2$;

R_1, R_2 and R_3 are independently H, alkyl or cycloalkyl;

A is hydrogen, alkyl, hydroxyalkyl, alkoxyaokyl, or OR';

B is hydrogen, alkyl, hydroxyalkyl, alkoxyaokyl, or OR";

R' and R" are independently hydrogen, alkyl, aralkyl, carbamoyl; alkyocarbamoyl, dialkylcarbamoyl, alkylcarbonyl, alkoxycarbonyl, aralkoxycarbonyl; aryloxycarbonyl, or, when A or B are OR' and OR", respectively, R' and R" together may form



where R_c is hydrogen or alkyl,

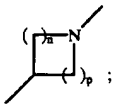
where R_d and R_e are independently hydrogen, alkyl, or together with the carbon atom to which they are attached may form a 1,1-cycloalkyl group;

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable pro drug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

2. A compound according to claim 1 wherein K is N;

T is hydroxymethyl or methoxymethyl;

A and B are hydroxy;



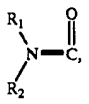
and n+p is 3 or 4;

or a pharmaceutically acceptable salt thereof, a pharamaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

3. A compound according to claim 2 which is (2R,3R,4S,5R)- 2-hydroxymethyl- 5-[6-[1-(5-chloropyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3S,4R,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(R)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-bromopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-(6-(1-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino)-purin-9-yl) tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(5'-trifluoromethyl-3,4,5,6-tetrahydro-2H-[1,2']-bipyridinyl-3-yl)-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl)-5-[6-(phenylpyrrolidin-3(S)-ylamino)-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-(1-pyridin-2-ylpyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-thiophen-2-ylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(5-methylmercaptopyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-hydroxymethyl-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3-ylamino]-purin-9-yl]-tetrahydrofuran-3,4-diol, (2R,3R,4S,5R)-2-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]tetrahydrofuran-3,4-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)piperidin-4-yl]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3S)-pyrrolidin-3-ylamino)-purin-9-yl] cyclopentane-1,2-diol dihydrochloride, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(R)-ylamino]-purin-9-yl]cylcyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3R)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-

3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chloropyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(pyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(quinolin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-S-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4,5-bistrifluorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-phenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4-[3(S)-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino]pyrrolidin-1-yl]benzonitrile, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(isoquinolin-1-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-bromoquinolin-2-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(4-chlorophenyl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-3-[6-[1-(3-chloro-5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R, 2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R, 5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S, 3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chlorpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylphenyl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1, 2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-phenylpyrrolidin-3-(S)-ylamino)-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino]purin-9-yl]5-hydroxymethylcyclopentane-1,2-diol, or (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino) purin-9-yl]5-methoxymethylcyclopentane-1,2-diol; or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

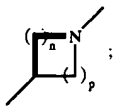
4. A compound according to claim 1 wherein Q is CH_2;



wherein R_1 is H and R_2 is lower alkyl;

K is N;

A and B are hydroxy;



X is

and n+p is 3 or 4;

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

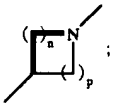
5. A compound according to claim 4 which is (1S,2R,3S,4R)-2,3-dihydroxy-4-[6-[1-(5-trifluormethylpyridin-2-yl) pyrrolidin-3-ylamino]-purin-9-yl]cyclopentanecarboxylic acid ethylamide, 5'-N-[1(S)-methylpropyl]-N6-[1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl]carbocyclic adenosine-5'-uronamide, or 5'-N-[1(R)-methylpropyl]-N6 [1-(5-trifluoromethylpyridin-2-yl)-pyrrolidin-3-(S)-yl] carbocyclic adenosine-5'-uronamide; or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

6. A compound according to claim 1 wherein Q is CH_2;

K is N;

T is hydroxymethyl or methoxymethyl;

A and B are hydroxy;



X is

and n+p is 3 or 4;

or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

7. A compound according to claim 6 which is (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)piperidin-4-yl]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3S)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol dihydrochloride, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-nitrophenyl)pyrrolidin-3-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(R)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1S,2R,3R,5R)-3-hydroxymethyl-5-[6-((3R)-pyrrolidin-3-ylamino)-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3 (S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,

2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chloropyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(pyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(quinolin-3-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-S-(4-nitrophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4,5-bistrifluorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-1447 3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(phenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, 4-[3(S)-[9-(2,3-dihydroxy-4-hydroxymethylcyclopentyl)-9H-purin-6-ylamino]pyrrolidin-1-yl]benzonitrile, (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(isoquinolin-1-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-bromoquinolin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(4-chlorophenyl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-[1-(3-chloro-5-trifluoromethylpyridin-2-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(6-methoxypyrimidin-4-yl) pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(6-chloropyridazin-3-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-trifluoromethylphenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-bromopyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(5-chlorpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylphenyl)pyrrolidin-3(S)-ylamino]-purin-9-yl] cyclopentane-1,2-diol, (1R,2S,3R,5R)-5-(-[6-[1-(4-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-methoxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-5-[6-[1-(3-chlorophenyl)-pyrrolidin-3(S)-ylamino]-purin-9-yl]-3-hydroxymethylcyclopentane-1,2-diol, (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-phenylpyrrolidin-3-(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol, (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-hydroxymethylcyclopentane-1,2-diol, or (1R,2S,3R,5R)-3-[6-(1-benzyl-pyrrolidin-3(S)-ylamino)purin-9-yl]5-methoxymethylcyclopentane-1,2-diol; or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

8. A compound according to claim 6 which is (1R,2S,3R,5R)-3-hydroxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol or (1R,2S,3R,5 R)-3-hydroxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

9. A compound according to claim 6 which is (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(5-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol or (1R,2S,3R,5R)-3-methoxymethyl-5-[6-[1-(4-trifluoromethylpyridin-2-yl)pyrrolidin-3(S)-ylamino]-purin-9-yl]cyclopentane-1,2-diol or a pharmaceutically acceptable salt thereof, a pharmaceutically acceptable prodrug thereof, an N-oxide thereof, a hydrate thereof or a solvate thereof.

10. The compound of claim 1, wherein K is an N-oxide.

11. A composition for treating a cardiovascular disease marked by hypertension or myocardial ischemia, for ameliorating ischemic injury or reducing myocardial infarct size consequent to myocardial ischemia, or for reducing lipid levels, triglyceride levels or cholesterol levels in a mammal, said composition comprising an effective amount of a compound according to claim 1 and a pharmaceutically acceptable carrier thereof.

12. A method for ameliorating ischemic injury in a patient in a patient suffering therefrom or reducing myocardial infarct size consequent to myocardial ischemia in a patient suffering therefrom, comprising administering to said patient an effective amount of a compound according to claim 1.

13. A method for treating a patient suffering from myocardial ischemia, comprising administering to said patient an effective antiischemic amount of a compound according to claim 1.

14. A method for treating a patient suffering from hyperlipidemia or hypercholesterolemia, comprising administering to said patient an effective antilipolytic amount of a compound according to claim 1.

15. A method for treating a patient suffering from hypertension, comprising administering to said patient an effective blood pressure lowering amount of a compound according to claim 1.

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